Investigations on the behavior of the carbamate insecticide pirimicarb and the thiocarbamate herbicide triallate and their metabolites in soil

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Wang, W., Kreuzig, R. and Bahadir, M. (1998): Laboratory lysimeter experiments on leaching of triallate and its metabolite 2,3,3-trichloro-prop-2-en-sulfonic acid in soil. Fresenius Envir. Bull., 7, 627-634.

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Abbreviations

amu	atomic mass unit
A.S.	active substance
BBA	Federal Biological Research Center for Agriculture and Forestry
C_{org}	organic substance described as carbon content
DAD	diode array detector
DFG	German Research Society
DT_{50}	disappearance time for 50 % of A.S. applied
ECD	electron capture detector
EI	electron impact ionization
eV	electron volt
FID	flame ionization detector
GC	gas chromatography
GPC	gel permeation chromatography
HP	Hewlett-Packard
HPLC	high performance liquid chromatography
I.D.	inner diameter
IUPAC	International Union of Pure and Applied Chemistry
KAS	cold injection system
K_d value	soil/water distribution coefficient
MS	mass spectrometry
MSD	mass selective detector
NCI	negative chemical ionization
n.d.	not detected
NK	Neuenkirchen (investigation site)
NPD	nitrogen-phosphorus-selective detector
n.q.	not quantified
NW	Nienwohlde (investigation site)
PMC	pirimicarb
PMC-D	pirimicarb-desmethyl
PMC-DF	pirimicarb-desmethylformamido
RIC	reconstructed ion current
RP	reversed phase

SAX	strong anion exchange
SIC	Sickte (investigation site)
SIM	selected ion monitoring
SCP	Special Collaborative Program
SFE	supercritical fluid extraction
SPE	solid phase extraction
TCPSA	2,3,3-trichloro-prop-2-en-sulfonic acid
TLC	thin layer chromatography
TRI	triallate
TSP-LC/MS	thermospray liquid chromatography-mass spectrometry
UV/VIS	ultraviolet/visible

1 Introduction

1.1 Application of pesticides in agriculture

Application of plant protection products in the modern agricultural practice is important for the control of a large number of weeds and pathogens to improve crop yields and to guarantee food quality. The loss of crop yields in 1986 through weeds and diseases was estimated to be about one third of the potential harvests worldwide (Thier and Frehse, 1986). As world populations further grow, the supply of enough food by increasing agricultural productivity will become a challenging task, at least in developing countries. In these countries, more and more farmland is being lost as a result of increasing demand for housing and industrial development. Thus, a global situation has developed in which the availability of prime agricultural land is decreasing and the total human population is increasing. To deal with this dilemma, an increase of agricultural productivity on existing farmland has become one of the important measures. Intensive and extensive use of pesticides is an inevitable response to this demand.

The situation is different in industrialized countries. Production of high quality food with low costs and high profit is of important economic interest for agricultural production in North America and Europe. This consequently depends on a low input of labor and a heavy use of machinery and chemicals. Pesticides, especially herbicides, are an important part of the chemicals used. The growth of food production by 34 % from 1951 to 1966 resulted in a 300 % increase of pesticides application around the world (Bahadir et al., 1992). Alone in Germany, 1144 registered pesticides at a total amount of 34 531 t A.S. were used in 1995; among them near 50 % were herbicides, followed by fungicides accounting for about 30 %. The rest was insecticides and growth regulators (Schmidt, 1998). This corresponded to an average annual application rate of 2 kg A.S./ha for agricultural land in Germany.

It is now well recognized that an excessive and indiscriminate use of pesticides can lead to ecological imbalances. After the early triumph of pesticides in the agricultural production, some persistent pesticides and/or their degradation products have been found to accumulate and transport between different environmental compartments, leading to contamination of atmosphere, ground- and surface water, soils as well as foods. For example, the drift of

pesticides during applications as well as the post-applying volatilization from soil or plant surfaces is responsible for air pollution. This has been evidenced by the detection of pesticides in rain water (Scharf et al., 1992; Siebers et al., 1994; Dörfler and Scheunert, 1997). Moreover, pesticides adsorbed on tiny air-suspended particles can be transported to a long distance in the atmosphere and deposited from there to soil, water or plant surfaces, causing additional contamination of these environmental compartments. The ubiquitous appearance of some persistent pesticides like the well-known insecticide DDT and its derivatives is an evidence of such phenomena. Numerous investigations have proved the existence of various pesticides and their degradation products in ground water or even drinking water (Bahadir et al., 1992; Hässelbarth, 1987; Cohen et al., 1984). The long-term extensive triazine application in maize production has resulted in a repeated appearance of this group of herbicides in ground water (Dörfler et al, 1997a). Thus, there have been growing concerns about the environmental and human health impact of pesticides. However, agricultural production without agrochemical is impracticable as long as there are no economically realistic alternatives available for crop protections. Therefore, pesticide bans have been both praised as far-sighted and damned as near-sighted.

Based on knowledge available, legislation regulating pesticide registrations, their application rates and ranges has been established in many countries and constantly updated as our understanding of the behavior of pesticides in the environment increases. Maximum tolerable concentrations have been set for pesticide residues in foods and drinking water. For example, to ensure a long-term ground water and drinking water quality, the drinking water regulation in most countries of the European community sets a limit of 0.1 µg/L as the maximum levels for individual pesticide and 0.5 µg/L for the sum of all pesticides (TVO, 1986).

Alternatives to the use of chemical pesticides are being searched for and efforts are being made to perform more aimed and controlled uses of pesticides. Nowadays, the concept of an integrated pesticide management (Burth et al., 1994), in which pesticides are considered only to be used to support biological, biotechnological and cultural measures, has already been accepted as the better alternative to the extensive and indiscriminate use of pesticides in the past. Pesticides of environmental risk have been banned from the market. In addition, new kinds of pesticides with less application rate but higher biological efficacy are continuously being developed. These efforts are reflected by the fact that from 1991 to 1995 the annual application of pesticides in most countries of the European community has continuously

reduced by 40 % (Anonymous, 1998). However, it should be mentioned that the reduced pesticide application does not mean a reduced risk potential for the environment. The higher biological efficacy of new pesticides must be taken into consideration by the risk evaluation. They present new challenges for their analytical monitoring in the environment.

To estimate the ecological impact of pesticides for the protection of environment and human health, a deep understanding of pesticides' behavior in different environmental compartments is an essential prerequisite. As depicted in Figure 1.1, pesticides undergo a series of reactions and transports in the environment, where soil takes a central position when considering the fate of the pesticides.

1.2 Behavior of pesticide residues in soil

Soil is important for the agricultural production and for the preservation of ground water quality. In comparison with other environmental compartments, soil has relatively high tolerance against natural and anthropogenic contamination. This is due to its inherent chemical and biological degradation processes that lead to an elimination of contaminants and counteract their accumulation in soil. However, excessive and indiscriminate use of pesticides can still cause damages to soil fauna and flora and result in loss of soil quality. In this respect, the application of pesticides should have no sustained impact on the ecological balance. This requirement is clearly expressed in the soil protection concept of the German Federal Government as well as in the legislation for cultural plant protection (Pflanzenschutzgesetz, PflSchG, 1998). Accordingly, substantial information about the behavior of pesticides in soil must be provided for the assessment within the context of pesticide registrations, as demanded and regulated by official guidelines (Schinkel et al., 1986; BBA, 1986; BBA, 1990). The input and output of pesticides in the different environmental compartments (e.g. soil, water, and atmosphere) should be closely monitored.

Contamination of soil by pesticides is inevitable. Pesticides arrive in soil through intended soil applications such as pre-emergence or post-emergence applications of some herbicides and insecticides, but also through undesired events. During foliar-applications of insecticides or fungicides, some active substances can unavoidably reach soil surfaces and contribute to the undesired soil contamination. Additionally, pesticide residues can be washed off from treated plant surfaces by precipitation events. They may fall from atmosphere as vapors or

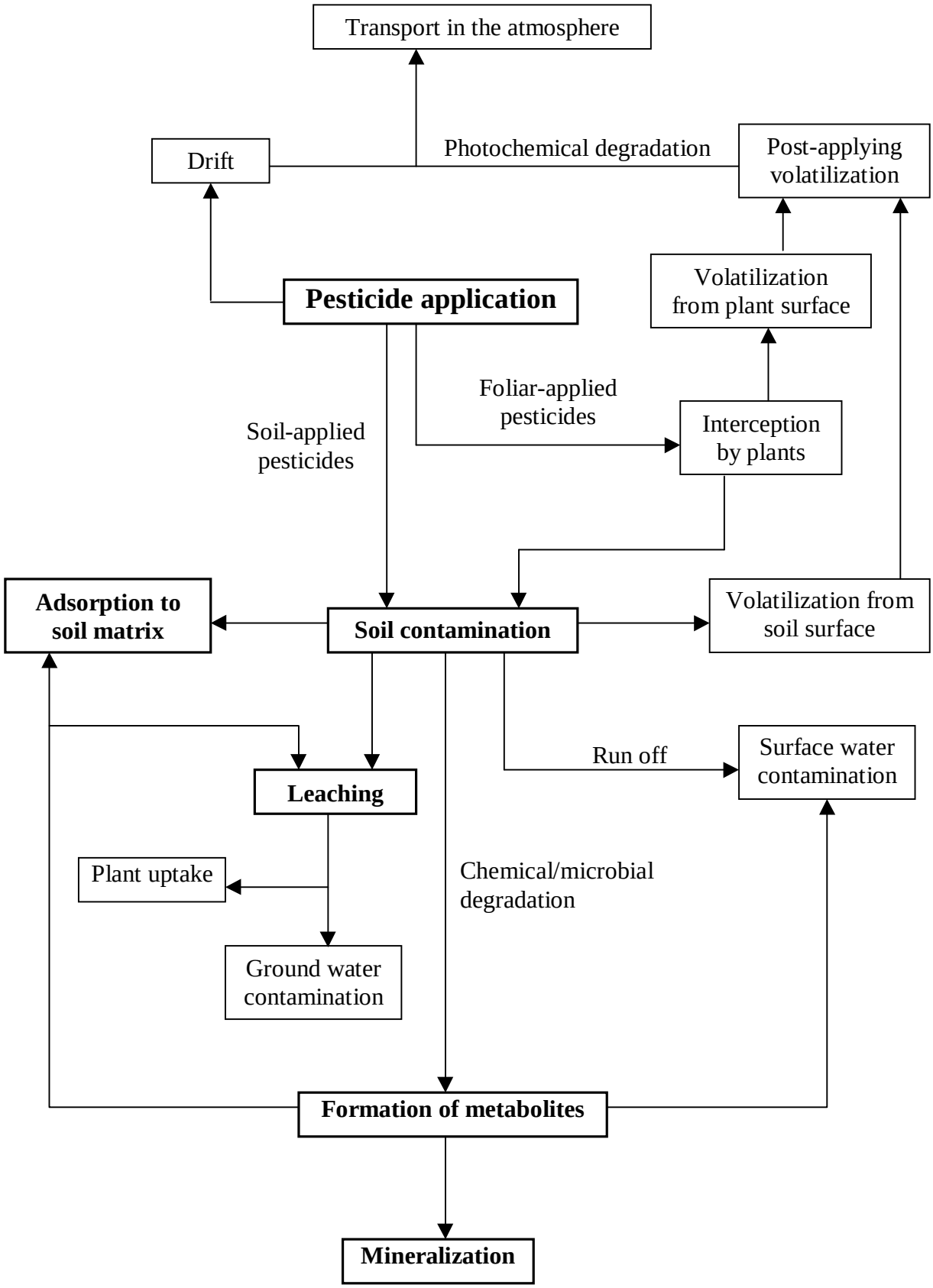


Figure 1.1 Behavior of pesticides in the environment (according to Kreuzig, 1994)

particle-associated by wet and dry deposition. Soil incorporation of pesticide contaminated plant residues is another source of soil pollution. Thus, soil is a sink for pesticides applied.

Pesticide residues undergo a series of complex physical, chemical, and biological processes in soil. They are often a combination of processes such as chemical and biological degradation, adsorption and desorption, leaching, as well as conversion to non-extractable residues. The fate of a given pesticide in soil depends on its physical-chemical properties and the soil properties. Numerous factors of environment and agricultural practice affect the behavior of pesticide residues as well.

Degradation and mineralization

After a pesticide applied has achieved its biological effect, its is desired that its residues should be rapidly degraded and not accumulated in soil for the long-term protection of soil qualities. This requires that pesticide residues should exist in bioavailable form, i.e. solved in soil solutions or weakly sorbed to soil matrix.

Complex soil-inherent chemical and biological transformation processes are responsible for the degradation of pesticides and the reduction of their accumulation in soil (Thier and Frehse, 1986; Bahadir et al., 1992). Chemical transformation processes consist of reactions of different mechanisms such as oxidation, hydrolysis and UV-induced photochemical reactions. Biological transformations of pesticide residues are usually non-specific enzyme-catalyzed degradations by microorganisms existing in soil. Especially enzyme-catalyzed oxidation processes are the most significant degradation processes in soil. Metabolites are formed as the products of such co-metabolic reactions. They can undergo further degradation reactions until the mineralization to H_2O and CO_2. However, total mineralization is rarely achieved in soil. Due to the persistence of pesticides and/or their metabolites, mineralization of pesticide residues usually only happens to a certain degree. For pesticides registrations, information on their persistence such as DT_{50} or DT_{90} values (time for the disappearance of 50 % or 90 % of the initially applied pesticide amount) are important assessment criteria. Lower persistence (DT_{90} value ≤ 100 days) is a prerequisite for the application permission (Schinkel et al., 1986; Kloskowski et al., 1992a).

Successive degradation of pesticide residues can lead to the formation of different kind of metabolites, which should also be considered as environmental contaminants as long as they are not subjected to further degradation. Sometimes the degradation products are even biologically more active than their parent compounds (bioactivation). Their behavior in soil may be completely different to their parent compounds. Knowledge about the sort and amount of degradation products and their behavior is thus, important in view of their ecotoxicological effects, their bioavailability to crops and, at last but not least, their higher leaching potential in soil, especially when considering the more polar properties of most degradation products in comparison with their parent compounds. Hence, simultaneous investigations on the behavior of pesticides and their degradation products are essential and required for the assessment in the registration procedures.

Formation of non-extractable residues

With the aging of pesticide residues in soil, adsorption of pesticides and their degradation products on the soil matrices becomes stronger, leading to the formation of non-extractable residues. Non-extractable residues are defined as "chemical substances, i.e. pesticides or their degradation products, formed after applications in accordance to good agricultural practice, which cannot be extracted with conventional residue analytical methods without significant changes of their chemical properties" (IUPAC, 1984). Therefore, non-extractable residue is a relative definition and can be varied between different analytical methods (Agnihotri and Barooah, 1994). According to the present knowledge, following mechanisms are involved in converting pesticide residues to non-extractable residues in soil: incorporation into the structure of clay particles; non-covalent incorporation into the space of humic substances through hydrogen bond, van der Waals forces or coulomb interaction; covalent binding to monomer-precursors of humic substances (Bahadir et al., 1992).

As long as pesticide residues remain immobilized, they have no adverse effect on the soil quality. However, processes of pesticide fixation can be reversible due to the permanent microbial breakdown and reconstruction processes of the humus. Non-extractable residues could be, thereafter, continuously released in low amounts and become bioavailable again for plant’s uptake and for further degradation and transportation. Consequently, bound residues have significant long term effect on the soil quality and should be constantly observed in case of possible later appeared mobility and bioavailability. Traditionally, ^{14}C-labeled pesticides

are used for the investigation on non-extractable residues and mineralization of pesticides, to gain detailed mass balance information of an applied pesticide. Information about non-extractable residues and mineralization is required for pesticides registrations (Kloskowski et al., 1992a)

Adsorption-desorption and leaching

Adsorption and desorption processes substantially affect the behavior of pesticides in soil. They determine the bioavailability, phytotoxicity, persistence, and mobility of pesticides. Adsorption can lead to an accumulation of pesticides and a decrease of pesticide concentrations in soil solutions, resulting in diminished bioavailability, reduced degradation and mobility. Through desorption pesticides become available for processes such as further chemical and biological degradations, uptake by plants, transport with surface water, distribution in the soil and leaching into ground water (Dörfler et al., 1997b).

Pesticide residues can be principally transported in soils in every direction, but leaching, i.e. downward movement through mass flow (convection) by percolation, is predominant in the humid climate areas like in the Middle Europe. Beside mass flow mechanism, transport on colloidal particles as well as the so called preferential flow (macro-pore flow or bypass flow) represent additional possible mechanisms of pesticide transport in soil (Nordmeyer et al., 1993; Nordmeyer and Aderhold, 1995; Dörfler et al., 1997b). Preferential flow indicates the fast movement of pesticides with water through favorable flow ways of geogenic, pedogenic, and biogenic origins such as soil crevices and earthworm tunnels. In comparison with mass flow, preferential flow is a rapid but short-time phenomenon. Because of the variable and complex nature of soils, accompanied by a number of adsorption-desorption and degradation processes, a quantitative description of the preferential flow for pesticide transport in soil is very difficult and not yet completely understood (Demuth and Hiltpold, 1993).

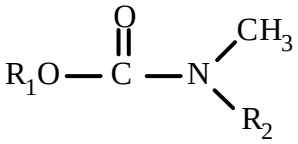
Leaching potential of a pesticide is influenced by a number of factors, namely pesticide properties, structures and properties of soils, soil microbial activities, hydrogeological specialties and climate conditions (Bailey and White, 1970; Adams, 1973; Herzel, 1987; Nordmeyer and Pestemer, 1989; Edwards et al., 1992; Pestemer, 1993; Matthess, 1993; Geiselhart, 1994). Additionally, agricultural practices like tillage and irrigation, especially the wide spread mono-culture of modern agricultural productions with extensive application of

the same pesticides, may result in a long-term accumulation of pesticide residues and an increased leaching potential into deeper soil layers. Because of the considerably lower microbial activities under the root zone, biological degradations are notably limited. This means a higher persistence of pesticide residues and a greater risk of ground water contamination (BBA 1986; Nordmeyer and Pestemer, 1989). Furthermore, some degradation products are more polar and water soluble than their parent compounds, possessing much higher leaching potential. For the evaluation of leaching behavior and the risk of ground water contamination, degradation products of pesticides should be, therefore, taken into account. According to the BBA-guidelines, potential ground water contamination exists, when one of the following criteria is fulfilled by pesticides and/or their degradation products: water solubility > 30 mg/L; sorption constant (K_{oc}) < 500, soil/water distribution coefficients (K_d) < 10 and DT_{50} value > 21 days. Thus, information about the leaching behavior of pesticides should be presented for the evaluation in registration procedures (BBA, 1986; BBA, 1990; Kloskowski et al., 1992b). In such case, lysimeter experiments with undisturbed soil monoliths are required to be carried out, taking into consideration of the local field conditions such as precipitation events, soil temperature and soil moisture.

2 Characterization of pesticides and soils

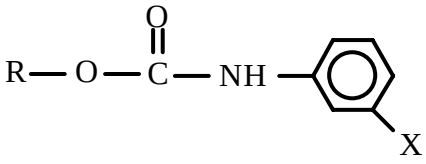
Derivatives of the carbamino acid are a large group of chemical pesticides with a wide spectrum of biological actions depending on the different substituted functional groups in their structures. They represent a variety of carbamate fungicides, herbicides and insecticides. The basic structures of herbicides and insecticides are shown in **Figure 2.1.**

Insecticide



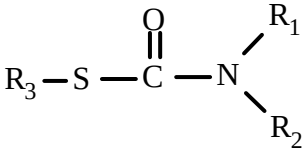
R_1 = aryl, heteroaryl, oxime; R_2 = H or CH_3

Herbicide



R = Alkyl; X = H or Cl

N-arylcarbamate



R_1, R_2, R_3 = Alkyl

S-alkylthiocarbamate

Figure 2.1 Basic structures of carbamate insecticides and herbicides (according to Thier and Frehse, 1986)

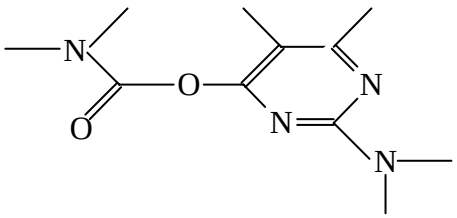
Carbamate insecticides play an important role in controlling insect pests. They can be generally applied where the insects have already become resistant against organophosphorus insecticides (Thier and Frehse, 1986). Carbamate insecticides have similar physiological effects as organophosphorus insecticides, namely inhibition of cholinesterase, but are generally better degradable and so have lower adverse environmental impact. Carbamate pesticides investigated in this work are the insecticide pirimicarb (2-dimethylamino-5,6-dimethylpyrimidine-4-yl dimethylcarbamate), an important representative of N,N-

dimethylcarbamate insecticides, and the herbicide triallate (S-2,2,3-trichloroallyl-N,N-diisopropylthiocarbamate), a representative of S-alkyl thiocarbamates herbicides.

2.1 The insecticide Pirimicarb

Technical information and physical-chemical properties (IVA, 1990, Perkow, 1994)

Common name	pirimicarb
Trade name	Pirimor®
Commercial products	Pirimor-granulate to solve in water (active ingredient 50 %)
	Pirimor smoking box (10 %), ICI
Active substance	2-dimethylamino-5,6-dimethylpyrimidine-4-yl dimethylcarbamate
CAS-No.	23103-98-2
EG-No.	006-035-00-8
Chemical formula	$C_{11}H_{18}N_{14}O_2$
Molecular weight	238.3 g/mol
Chemical structure	



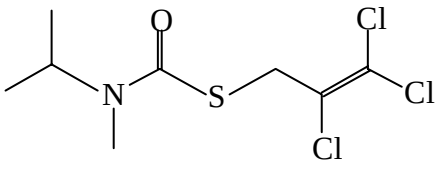
Melting point	90,5 °C
Vapor pressure	5×10^{-3} Pa at 20 °C
Solubility (in g/100 g at 20 °C)	water: 0.300 (pH 7.4)
	ethanol: 25
	methanol: 23
	Xylol: 29
n-octanol/water partition coefficient (log Po/w)	1.8

Agricultural application

Pirimicarb is a fast-acting selective aphicide of moderate mammalian toxicity. It has been proved to be effective against organophosphorus-resistant aphids through contact and translaminar actions (Proctor and Baranyovits, 1969; Rexilius, 1983; Entwistle, 1989; Worthing, 1987). Pirimicarb is taken up by the roots and translocated through the xylem and does not penetrate into the fruits (FAO/WHO, 1977). It has been reported to be especially suitable for aphid controls at high ambient temperature (Cabras et al., 1990). Its highly selective action makes it widely employed in integrated control programs. Since the 70's pirimicarb has been widely applied for controlling aphids in various kinds of crops, such as cereals, sugar beets, potatoes, fruits, and vegetables. Depending on the crops, the application rates varied from 200 to 500 g A.S./ha and the waiting time before harvest is 7 to 35 days. Pirimicarb is one of the important insecticides used in Germany.

2.2 The herbicide triallate

Technical data and physical-chemical properties (IVA, 1990, Perkow, 1994)

Common name	triallate
Trade name	Avadex BW®
Commercial products	Avadex BW (400g active ingredient /L) solved in heavy aromatic naphtha
Active substance	S-2,3,3-trichloroallyl-N,N-diisopropyl-thiocarbamate
CAS-No.	2302-17-5
EG-No.	006-039-00-X
Chemical formula	$C_{10}H_{16}C_{l3}NOS$
Molecular Weight	304.7 g/mol
Chemical structure	
Appearance	colorless crystal (pure),

	light brown oily liquid (technical)	
Melting point	29 - 30 °C	
Boiling point	117 °C at 40 Pa	
Vapor pressure	2.76×10^{-2} Pa at 25 °C	
Solubility (in g/100 g at 20 °C)	water:	0.0004
	acetone:	> 20.0
	dichloromethane:	> 20.0
	ethyl acetate:	> 20.0
	hexane:	2 - 5
	methanol:	> 20.0
	toluene:	> 20.0
n-octanol/water partition coefficient (log Po/w)		4.55

Agricultural application

Triallate is widely used as selective pre-emergence herbicide for the control of diverse monocotyledons such as wild oat, slender foxtail and wind bend grass in different crops like cereals, oilseeds and sugar beets. The phytotoxic action of triallate in soil is attributed to vapor absorption by shoots of susceptible seedlings (Banting, 1967; Miller and Nalewaja, 1976). It is registered for soil application at a rate of 1.2 - 1.7 kg A.S./ha depending on the crops. Generally, triallate is spray-applied to soils as emulsified Avadex BW® (400 g A.S./L water), and is recommended to be incorporated immediately into the superficial 0-5 cm soil layers to minimize its losses via volatilization.

2.3 Soil properties

The complex fate of a pesticide in soil is not only determined by its physical-chemical properties, but is also influenced by soil properties such as C_{org}, pH, soil texture and microbial biomass. Information on pesticide behavior in soils of different properties is also required in the registration procedures for assessment. In this work, different soil types were used for the investigation purpose. The properties of soils investigated are presented in **Table 2.1**.

The two SCP investigation sites Neuenkirchen (NK) and Nienwohlde (NW), located in different areas of Lower Saxony, were chosen in this work for investigations on the behavior

of pirimicarb and two of its corresponding metabolites pirimicarb-desmethylformamido (PMC-DF) and pirimicarb-desmethyl (PMC-D). The NK-soil is clayey silt and the NW-soil silty sand. Soil samples taken from superficial 0-5 cm soil layers at both investigation sites were used for fortification experiments and laboratory batch experiments. Field experiments were carried out by foliar-application of pirimicarb to the sugar beets at the two test sites. In addition, direct soil-application was performed in Neuenkirchen as well.

Table 2.1 Soil properties at the investigation sites Neuenkirchen, Nienwohlde, Sickte and the Federal Biological Research Center for Agriculture and Forestry (BBA), Lower Saxony, Germany (W_k: maximal water capacity)

Investigation sites	Neuenkirchen [NK]	Nienwohlde [NW]	Sickte [SIC]	BBA
Soil texture	Clayey silt	Silty sand	Loamy sand	Loamy sand
Sand [%]	2	76	49	55
Silt [%]	80	19	40	34
Clay [%]	18	5	11	11
$C_{org.}$ [%]	0.97	1.52	1.0	1.2
pH [0.01 M $CaCl_2$]	7.1	5.3	6.1	6.6
W_k[%]	33	27	30	-

Soils sampled from the investigation sites in Nienwohlde (NW), Sickte (SIC) as well as in the Federal Biological Research Center for Agriculture and Forestry (BBA), all located in Lower Saxony, were used for investigations on the behavior of triallate and its major metabolite 2,3,3-trichloro-prop-2-en-sulfonic acid (TCPSA) in soil.

Soil samples taken from superficial 0-5 cm soil layers at the investigation sites in Nienwohlde (NW) and Sickte (SIC) were used in fortification experiments for the analytical method development. For laboratory batch experiments the loamy sand soil from Sickte (SIC-soil) was used. Undisturbed soil monoliths (0-30 cm) sampled at the investigation site BBA were used for laboratory lysimeter experiments.

3 Stand of research and scope of this work

3.1 Degradation behavior

Pirimicarb

Pirimicarb disappears very rapidly in plants, animals and soils by volatilization, metabolic, and photochemical degradation. Two pathways have been described for the degradation of pirimicarb, as shown in **Figure 3.1** (FAO/WHO, 1977):

- successive N-dealkylation of the 2-dimethylamino group to form the carbamate-containing metabolites II-IV;
- decarbamoylation of the carbamate moiety with further N-dealkylation of the 2-dimethylamino group to have the hydroxypyrimidines V-VII.

Extensive studies on the dissipation of pirimicarb in plants have been carried out by many groups (FAO/WHO, 1977; Wüest and Meier, 1983; Szeto et al., 1984; Szeto et al., 1985; Magalhâes et al., 1989; Cabras et al., 1990; Cabras et al., 1995). The major degradation products were identified as the carbamate-containing metabolites II (PMC-DF) and III (PMC-D), while the carbamate-containing metabolite IV and the hydroxypyrimidines V-VII are the minor products. However, pirimicarb degradation varies largely with crop types and weather conditions. For example, pirimicarb metabolites, determined mainly as the carbamate-containing 2-methylformamido (PMC-DF) and 2-methylamino derivatives (PMC-D), have been reported to reach amounts more than that of the parent compound in some vegetables under field conditions (Szeto et al., 1984; Szeto et al., 1985; Magalhâes et al., 1989; Cabras, et al., 1990), but none of the metabolites (II-VII) was detected in field and greenhouse fruits (Cabras et al., 1995).

The principal degradation route of pirimicarb in animals was found to be hydrolysis of the carbamate moiety. The hydroxypyrimidines V-VII were considered to be the major degradation products, whereas the carbamate-containing metabolites were absent or in very low amount (FAO/WHO, 1979). A rapid and almost total metabolization of pirimicarb was found in rats after oral application (Perkow, 1994).

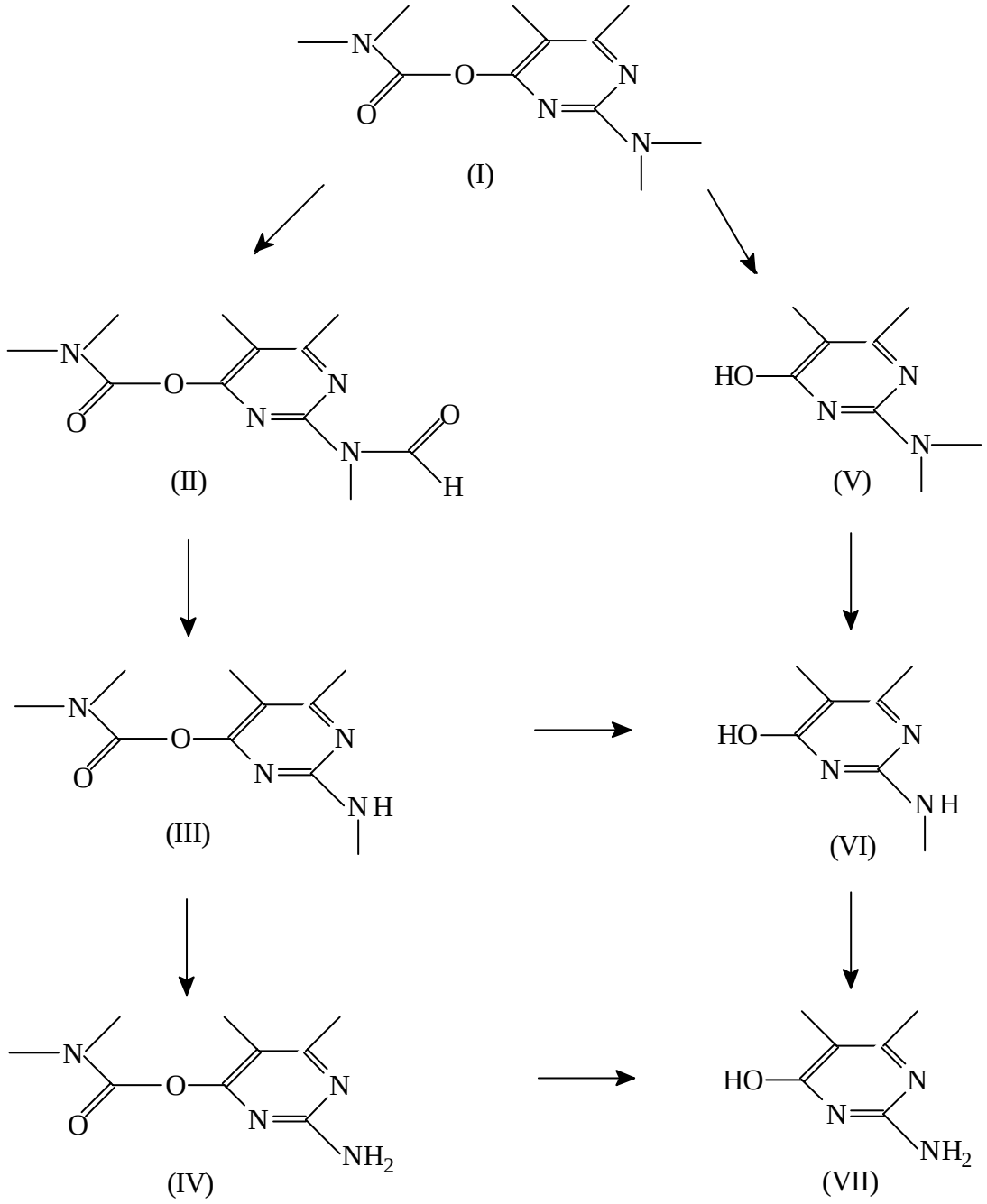


Figure 3.1 Pirimicarb and six of its metabolites:

(I) pirimicarb;

(II) 2-methylformamido-5,6-dimethylpyrimidine-4-yl dimethylcarbamate (PMC-DF);

(III) 2-methylamino-5,6-dimethylpyrimidine-4-yl dimethylcarbamate (PMC-D);

(IV) 2-amino-5,6-dimethylpyrimidine-4-yl dimethylcarbamate;

(V) 2-dimethylamino-5,6-dimethyl-4-hydroxypyrimidine;

(VI) 2-methylamino-5,6-dimethyl-4-hydroxypyrimidine;

(VII) 2-amino-5,6-dimethyl-4-hydroxypyrimidine.

Relatively little is known about the degradation of pirimicarb and the formation and behavior of its metabolites in soils. Degradation of pirimicarb in soil was reported as mainly through the hydrolysis of the carbamate moiety either by biological or chemical mechanisms, and the metabolites V, VI and III were the major products. Hill (1976) characterized the non-extractable residues of ^{14}C-labeled pirimicarb (ring-2-^{14}C and carbamate-^{14}C) in several sterile and non-sterile soils under controlled aerobic or anaerobic conditions. Pirimicarb was found to degrade most rapidly in alkaline soils. Both the degradation of pirimicarb and the formation of non-extractable residues contributed to the decline of pirimicarb. But no relationship was found between the rate of non-extractable residue formation and either the rate of pirimicarb degradation or the soil pH and organic matter content. In sterile soils both pirimicarb degradation and bound residues formation were considerably lower than their non-sterile equivalents, indicating that microbiological degradation of pirimicarb was the predominant way of pirimicarb dissipation in soil. Concerning the degradation products, it was merely mentioned by Hill (1976) that in the acetone/water extracts of an alkali soil and an acidic soil after 5 weeks' incubation, hydroxypyrimidine metabolites accounted for 8 % and 38 % of the radioactivity initially applied, respectively, while approximately 5 % and 1 % were identified as the sum of the carbamate-containing metabolites 2-methylformamido (PMC-DF) and 2-methylamino derivatives (PMC-D) in these two kind of soils. Concentration changes of these metabolites were not shown. Gottesbüren et al. (1992) found that concentration of pirimicarb in soil decreased to values below the determination limit 20 - 24 weeks after field applications to sugar beet and winter wheat. In addition, pirimicarb showed only a low leaching tendency into the 5-10 cm soil layers. Little is reported on the formation and the fate of the corresponding metabolites under field conditions.

Photochemical reactions were suggested to play an important role for the degradation of pirimicarb on plant and soil surfaces (Bullock, 1973; FAO/WHO, 1977; Kopf, 1992; Romero et al., 1994; Cabras et al., 1995). Metabolite V was found to be the major product of the photochemical degradation on soil surfaces, accounting for 84% of the total degradation. (Bullock, 1973; FAO/WHO, 1977). Kopf (1992) and Romero et al. (1994) identified PMC-DF and PMC-D as the major photo-degradation products in water, organic solvents and plant surfaces under natural and simulated sunlight. Additionally, Kopf (1992) suggested that not only biological but also photochemical degradation of pirimicarb should be taken into account, when considering the rapid disappearance of pirimicarb from plant surfaces. Cabras et al. (1995) carried out laboratory tests to evaluate the photo-degradation of pirimicarb as

well as its metabolites PMC-DF and PMC-D in Petri dishes and capped vials. While PMC-DF and PMC-D were already detected as pirimicarb photo-degradation products 30 min after the test beginning, about 20 % of PMC-DF and 80 % of PMC-D themselves were found to degrade in a 4 h test period. However, the authors pointed out that PMC-DF did not converted to PMC-D, as often reported in the past (FAO/WHO, 1977; Kopf, 1992). Furthermore, no peaks of the corresponding hydroxypyrimidines were detected by HPLC analysis as PMC-DF and PMC-D progressively degraded.

Triallate

Various investigations on the behavior of triallate in soils have been reported in the past years (Banting, 1967; Smith, 1969; Smith, 1970; Anderson and Domsch, 1980; Anderson, 1981; Smith and Muir, 1984; Cantwell et al., 1989; Gottesbüren et al., 1992). Because of its high volatility, losses of triallate from treated soils were considered to be due to the combination of volatilization and biological degradation (Grover et al., 1981; Banting, 1967). Volatilization of triallate increased with increased soil temperature (Grover et al., 1978). Indeed, triallate was detected in rain samples at a concentration range between 0.06 - 2.2 µg/L as a result of volatilization into the atmosphere immediately after soil applications in several places of Germany (Scharf et al., 1992). However, evaporation of triallate from treated soils varied strongly according to soil properties and weather conditions. Due to extensive adsorption of triallate to soils investigated in his study, Smith (1970) suggested that the volatilization loss of triallate was negligible even after heating of the soil to 50 °C for 28 days. Instead of that, microbial degradation was the prime factor contributing to the loss of triallate. Jury et al. (1980) simulated the vapor loss of soil-incorporated triallate in the presence and absence of evaporating water and found that vapor losses did not exceed 5 % of the amount applied. This was also proved by Anderson (1981). The major routes of triallate dissipation were, therefore, proposed to be microbial degradation and conversion to non-extractable soil residues. Volatilization played an insignificant role. Herzel and Schmidt (1987) found that volatilization of triallate reduced remarkably with increasing organic carbon-content in soil.

Although dissipation rate of triallate is strongly influenced by soil properties and weather conditions, triallate is regarded as a persistent herbicide in soils and its carrying-over from one vegetation period to the next has been often observed (Smith, 1970; Gottesbüren et al., 1992). On the other side, enhanced degradation of triallate was reported in soils that had

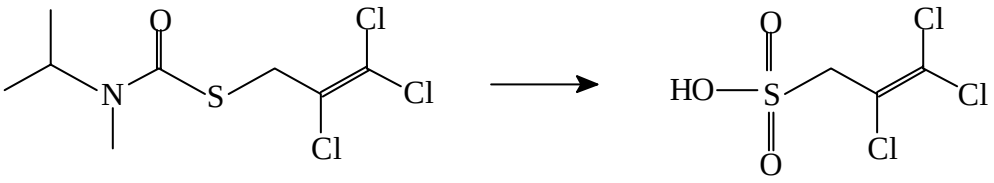
annually received 1.7 kg/ha of triallate for 23 years (Cotterrill et al., 1989). Degradation rate of triallate was found to increase with increasing soil moisture content but decrease with prolonged incubation (Smith, 1969, 1970; Anderson 1981). Soil incubations with allyl-2-^{14}C-triallate have been carried out under controlled experimental conditions and the distribution of radioactivity between extractable triallate and its degradation products, non-extractable residues and mineralization were characterized. Different results were obtained. While non-extractable residue was found to be the major radioactive component within 10 weeks of soil incubation by Anderson (1981), $^{14}CO_2$ was identified by Cantwell et al. (1989) as the major degradation product after 12 weeks incubation, accounting for over 50 % of the radioactivity applied. Besides, Cantwell et al. (1989) noted that extractable metabolites accounted for about 5 % of the original radioactivity. Under field conditions, Smith and Muir (1984) proved that only about 5 % of the radioactivity originally applied was in the form of degradation products, the majority of extractable radioactivity corresponded to the parent compound triallate. In addition, non-extractable residues increased steadily from 15 % to 30 % in the period from 45 to 95 weeks after the soil application of ^{14}C-triallate, while extractable residues decreased from 50 % to 16 % in the same period. However, none of the authors have identified any degradation products of triallate in soil.

Dissipation of triallate in plants has been reported to be faster than in soils. Cessna (1990) determined residues of triallate in pre-plant and pre-emergence treated triticale. He found that due to growth dilution and triallate metabolism, triallate residues in 5 to 6 leaf samples could hardly be determined. McMullan and Nalewaja (1991) investigated the influence of triallate absorption and metabolism on its tolerance in three sorts of wheat using ^{14}C-triallate at different temperatures. Metabolites were detected in shoot tissues at both 8 °C and 24 °C. Although metabolites concentrations were mentioned to have reached levels much higher than that of their parent compound at 24 °C, identification of the metabolites was not performed.

Metabolic fate of triallate as well as its dichloro-analogue diallate in rats has been extensively investigated and some metabolites formed have been identified (Hubbell and Casida, 1977; Schuphan and Casida, 1979; Marsden and Casida, 1982; Hackett, et al. 1990; Mair and Casida, 1991). More recently, a complete investigation on the metabolism of triallate in Sprague-Dawley rats was reported (Ridley et al., 1993; Nadeau et al., 1993; Hackett et al., 1993). Nadeau et al. (1993) identified twelve metabolites excreted by rats dosed with triallate. They suggested that triallate metabolism in rats proceeds mainly via three pathways: (1) S-

oxidation leading to sulfinic and sulfonic acids; (2) S-oxidation/hydrolysis/reduction leading to thiole derivatives; and (3) C-oxidation of the 2,3,3-trichloropropenethiol moiety. S-oxidation should represent the primary route of triallate metabolism in rats, resulting in the formation of the most abundant 2,3,3-trichloro-prop-2-en-sulfinic acid (26 %), and 2,3,3-trichloro-prop-2-en-sulfonic acid (TCPSA) (**Figure 3.2**), which was generally less than 5 % of the dosed triallate. TCPSA has been proved to be a metabolically inert strong acid. It migrated on high-voltage electrophoresis toward the anode at pH 1.0. It is the most polar metabolite of triallate with high water solubility. Hackett et al. (1993) found that the TCPSA level increased on prolonged storage of incubation samples, due to air oxidation or enzymatic conversion of the main metabolite 2,3,3-trichloro-prop-2-en-sulfinic acid. TCPSA was completely non-reactive with rat liver microsomes under oxidative conditions and did not undergo any reaction with glutathione (GSH).

Relatively little investigation has been undertaken on the determination of degradation products of triallate in soils or plants. According to our knowledge, merely Ebing and Schuphan (1978) have studied the metabolism of triallate in sugar beets and soils. They simply mentioned to have identified 2,3,3-trichloro-prop-2-en-sulfonic acid (TCPSA) by using thin layer chromatography (TLC) and gas chromatography with mass spectrometric detection (GC/MS), but without given any detailed information. TCPSA was described as an important and accumulated metabolite of triallate in sugar beets and soils. In contrast to triallate, TCPSA showed no phytotoxicity against weeds or wheat (Schuphan et al., 1977).



triallate TCPSA

Figure 3.2 An important metabolite of triallate: 2,3,3-trichloro-prop-2-en-sulfonic acid (TCPSA)

Triallate is non-polar and has very low water solubility. Residue of triallate was normally detected in the superficial 0-5 cm soil layers and negligible residue was found at the 5-10 cm soil level, indicating low leaching tendency of triallate under field conditions (Gottesbüren et

al. 1992). In a remarkable contrast to triallate, TCPSA is highly water-soluble and relatively stable in soil environment.

3.2 Analytical methods

Pirimicarb and its metabolites

As a commonly used insecticide, pirimicarb has been integrated into many pesticide multi-residue analytical methods like the DFG S19 multi-residue method (DFG, 1991a). It is routinely monitored in a broad variety of environmental samples, such as agricultural products and soils. In many of these multi-residue methods, conventional solvent extraction combined with liquid-liquid partition is used. One or more steps of extracts' clean-up are generally needed for sensitive determination (Wüest and Meier, 1983; Specht and Tillkes, 1985; DFG, 1991a; Luck et al., 1988; Mattern et al., 1990; Andersson and Pålsheden, 1991; Liao et al., 1991; Tuinstra et al., 1991a, 1991b; Ting and Kho, 1991; Bernal et al., 1992; Kadenczki et al., 1992; Nakamura et al., 1994). Solid phase extraction (SPE) has also been employed in some multi-residue methods, especially for the enrichment of pesticide residues from aqueous samples. RP-C_{18} SPE-cartridge is widely used, which normally gives comparable or even better recoveries for pirimicarb than solvent extractions (de la Colina et al., 1993; Bolygó et al., 1991; Schlett, 1991). Recently, approaches based on supercritical fluid extraction (SFE) have found increasing applications in pesticide multi-residue analysis (Khan, 1995; Koinecke et al., 1997). Alzaga et al. (1995) have tried to extract pirimicarb bound in soil with SFE. Sensitive determination of pirimicarb, for example 0.003 µg/L in fortified water samples (Bolygó et al., 1991) and 1 µg/kg dry soil (Gottesbüren et al., 1992), is promised in these multi-residue methods by gas chromatography with nitrogen-phosphorous-selective detection (GC/NPD) or mass spectrometric detection (GC/MS) or by reversed phase high performance liquid chromatography with ultraviolet detection (HPLC/UV or HPLC/DAD). Thin layer chromatography (TLC) is sometimes used in multi-residue methods as an additional confirmation (Gardyan et al., 1992; Zoun et al., 1989; Ambrus et al., 1981). Determination of pirimicarb by supercritical fluid chromatography (SFC) (Berry et al., 1986) as well as HPLC analysis with fluorometric detection after post-column photolysis of pirimicarb (Miles, 1988) were also reported.

Different approaches have been made for a simultaneous determination of residues of pirimicarb and its metabolites in agricultural products, soils and water samples. Most of them were focused on agricultural products like fruits and vegetables. Brauckhoff et al. (1987) analyzed residues of 15 methyl-carbamate insecticides and 4 metabolites in plant tissues including pirimicarb and its two carbamate-containing metabolites PMC-DF (II) and PMC-D (III) by GC/NPD without derivatization on a non-polar capillary separation column like DB-1. In some cases, PMC-DF was first converted to PMC-D under acidic conditions and determined after neutralization as the total amount of PMC-D (Bullock, 1973; Magalhâes et al., 1989; Szeto et al., 1984, 1985). Cabras et al. (1995, 1990, 1989) developed an analytical method for determining pirimicarb (I) and its 6 metabolites (II-VII, see **Figure 3.1**) based on a separated solvent extraction of I-IV with acetone/dichloromethane/sodium chloride solution and of V-VII with methanol or 1-butanol, followed by clean-up with NH_2-cartridge and analyzed with HPLC/UV. The metabolites IV-VII were, however, never detected in the plant samples investigated in their works. Thermospray liquid chromatography-mass spectrometry (TSP-LC/MS) was also tested for the analysis of hydroxypyrimidine metabolites of pirimicarb (Barcelo et al., 1991). Durand et al. (1991) and Honing et al. (1995) tried to determine pirimicarb and its hydroxypyrimidine metabolites V-VII in soil samples by HPLC/DAD as well as TSP-LC/MS, but without to mention whether the metabolites were detected in the soil samples or not. Using GC/NPD and GC/MS, only pirimicarb was determined by Honing et al. (1995). Some photo-degradation products of pirimicarb, mainly the metabolites II, III and V, were determined by HPLC/UV and confirmed by GC/MS, IR and NMR by Romero et al. (1994).

Triallate and its metabolite TCPSA

The analysis of triallate residues has been incorporated into numerous multi-residue methods for different environmental samples (Smith, 1974; Lee and Chau, 1983a, 1983b; Cessna et al., 1991a, 1987; Luck et al., 1988; Bruns et al., 1991; Schlett, 1991; Tuinstra et al., 1991a, 1991b; Bernal et al., 1992; Erdmann et al., 1992; Cairns et al., 1993; Olson et al., 1994; Volmer and Levsen, 1994; Volmer et al., 1994; Volmer, 1994; Butz and Stan 1995; Schülein et al., 1995). Like pirimicarb, triallate can be regularly monitored in plants, soils and sediments together with other pesticides using the DFG S19 multi-residue method (DFG, 1991a). In addition to the variety of solvent extraction methods available in the literature, solid phase extraction (SPE) has been often employed, as well. Schülein et al. (1995)

compared different solid phase extraction materials and techniques and proved that the most universally applied RP-C_{18} SPE-cartridge is most suitable for triallate extraction from aqueous samples. Supercritical fluid extraction (SFE) has also been successfully used to extract triallate and other pesticides from soil matrices (Koinecke et al., 1997). With three Cl atoms in its molecule triallate can be sensitively determined by GC/ECD, for example, in soils with a detection limit of 3 µg/kg (Gottesbüren et al., 1992).

Methods for single residue analysis have also been reported (Cook et al., 1982; Heras and Sanchez-Rasero, 1986; BBA, 1989b; Sanchez-Brunete et al., 1991). Triallate was extracted from soil samples with methanol, followed by clean-up on Sep-Pak C_{18} cartridge and determined by reversed phase high performance liquid chromatography with UV-detection (Heras and Sanchez-Rasero, 1986) or by gas chromatography with ion trap detection (Sanchez-Brunete et al., 1991). From drinking and percolate water, plants, and soils, triallate could be extracted with isooctane/sodium sulfate solution, followed by clean-up on Florisil column and quantified by GC/ECD. Triallate was also steam-distilled directly from aqueous suspensions of milk and plant samples, cleaned up on Florisil or Silica columns and then quantified by GC (Cook et al., 1982).

There are only a few analytical methods described for the determination of TCPSA. It has been reported to be isolated from rat urine samples using preparative HPLC and then analyzed by HPLC/UV directly or after the methylation with trimethyl orthoformate (Nadeau et al., 1993). Another analytical method was described for the determination of TCPSA in drinking water, in which TCPSA was ion pair extracted by dichloromethane with a phase transfer catalyst under acidic condition. Determination was carried out by GC/ECD, after the esterification of TCPSA with triethyl orthoformate. It was mentioned briefly that this method could be also used for the determination of TCPSA in other sample matrices, such as plant tissues, soils, and percolate water samples (BBA 1989a).

3.3 Scope of this work

It is clear that a deep and extensive understanding of the fate of pesticides and their residues is essential for the protection of environment and human health. In the past, researches have been mainly focused on investigating the active substances of plant protection products. With the extension of our knowledge about the behavior and significance of pesticides in the

environment, new aspects such as the formation and fate of pesticides degradation products are receiving increasingly attention. Furthermore, for a comprehensive evaluation of the ecological impact, investigations on the behavior of pesticide in and between different environmental compartments become also important. In view of these facts, the German Research Society's Special Collaborative Program (SCP 179) "Water and Matter Dynamics in Agro-Ecosystems" was established at the Technical University of Braunschweig. It aimed at an interdisciplinary research for a comprehensive evaluation of the dynamics of water and substances in selected agricultural ecosystems, which should, in one aspect, improve the estimation and prediction of eventual environmental problems of pesticide applications at present and in the future. A project, which focused on the investigation of the behavior of pesticides and their corresponding degradation products in soils, was incorporated into the Special Collaborative Program as a subproject (A 15). The work presented here was carried out within the frame of this project.

As the literature reviews carried out above, investigations on the carbamate insecticide pirimicarb and the thiocarbamate herbicide triallate in soils have been so far mainly focused on the pesticides themselves. The purpose of this work was to investigate the degradation behavior of pirimicarb and triallate in soils, with emphasis on the formation and fate of their metabolites, especially in comparison with the parent compounds. As metabolites are generally expected to appear at trace levels in soil, it is always a prerequisite that sensitive analytical methods must be adapted or developed for the investigations on pesticides' metabolites.

Cabras et al. (1995) have pointed out that the aphicide action could also be ascribed to the carbamate-containing metabolites 2-methylformamido (PMC-DF) and 2-methylamino derivatives (PMC-D) which should also be taken into account by the maximum residue limit (MRL) evaluation of pirimicarb. The appearance of these two carbamate-containing metabolites in soils was only briefly mentioned in a controlled soil incubation experiment (Hill, 1976). It was, therefore, desirable to get more information about the formation and behavior of these metabolites in soils, especially under field conditions.

Since no analytical methods were reported for the extraction of PMC-DF and PMC-D from soil matrices, it was planned to adapt the DFG S19 multi-residue method for a simultaneous determination of pirimicarb and the two metabolites. The reliability and sensitivity of the

analytical procedures should to be evaluated with fortification experiments. Recoveries and determination limits of the target compounds in soil samples should be determined.

Subsequently, the applicability of the analytical method adapted needed to be validated in laboratory batch experiments which were to be performed according to the BBA-guideline 4-1 (Schinkel et al, 1986). Information on the degradation behavior of pirimicarb should be gathered in two selected different soils, so that influences of soil properties on the degradation of pirimicarb and the appearance and concentration changes of the metabolites could be determined.

A main purpose of this work was to study the behavior of pirimicarb and the corresponding metabolites under field conditions during agricultural applications. Field experiments should be carried out to examine the transferability of laboratory results to the field application situations and to characterize the residue situations of pirimicarb and its metabolites under field conditions at the two SCP-investigation sites Neuenkirchen (NK) and Nienwohlde (NW), after foliar- or soil-applications of pirimicarb.

The soil/water distribution coefficients (K_d values) of pirimicarb and the two metabolites PMC-DF and PNC-D were to be determined by adsorption equilibrium studies for the evaluation of their adsorption behavior and leaching potentials in soils.

Because TCPSA has been suggested to be the major metabolite of triallate in soil (Ebing and Schuphan, 1978), it was reasonable to choose it in this work for the study of triallate degradation in soil.

One of the main objectives of this work was to develop a sensitive and effective analytical method for a simultaneous identification and quantification of the non-polar parent compound triallate and its highly water-soluble metabolite TCPSA in water and soil samples. To extract triallate and, especially, TCPSA from aqueous and soil samples, methods available should be tested at first, and new sample preparation method must be eventually developed. With three Cl atoms in their molecules, both compounds could be sensitively determined at trace levels by GC/ECD. However, TCPSA is too polar for direct gas chromatographic detection. A derivatization method should be, consequently, optimized to enable its sensitive determination with GC/ECD.

Then, the robustness of the new method needed to be validated by fortification experiments with spiked water and soil samples. Recoveries and determination limits were to be evaluated. Laboratory batch experiments should be carried out to confirm the appearance and concentration changes of TCPSA in soil samples fortified with triallate and incubated under controlled experimental conditions.

Because of the remarkable difference in the polarities of triallate and TCPSA, it is likely that TCPSA has a much greater leaching potential in soil than its parent compound, resulting in potentially ground water contamination. Therefore, it should be important and interesting to study the mobility of TCPSA in comparison with the parent compound triallate. A primary evaluation of their leaching potentials could be performed by the determination of soil/water distribution coefficients (K_d values). Furthermore, laboratory lysimeter experiments with undisturbed soil monoliths under experimental conditions similar to the field dynamic situations should be carried out to characterize the leaching tendency of both compounds into deeper soil layers.

4 Materials and methods

4.1 Chemicals and equipment

Reference chemicals

Reference chemicals used in this work are listed in **Table 4.1**.

Table 4.1 Pesticides and their metabolites

Substance	**Abbreviation**	**Company**
Pirimicarb	PMC	Ehrenstorfer, Augsburg
Pirimicarb-desmethylformamido **(10 ng/µl acetonitrile or toluene)**	PMC-DF	Ehrenstorfer, Augsburg
Pirimicarb-desmethyl (10 ng/µl acetonitrile or toluene)	PMC-D	Ehrenstorfer, Augsburg
Triallate (purity > 99.9%)	TRI	Monsanto, Belgium
2,3,3-Trichloro-prop-2-en-sulfonic acid (purity > 99.9%)	TCPSA	Monsanto, Belgium

Preparation of standard solutions

Stock standard solutions of pirimicarb, triallate, and TCPSA were prepared by solving 10.00 ± 0.10 mg of each analytical reference chemical with an appropriate solvent (acetone for pirimicarb, hexane for triallate and methanol for TCPSA) in a 10-mL flask to get a concentration of 1 µg/µL. These stock standard solutions were stored in a freezer at - 20°C.

Working standard solutions were freshly prepared by diluting the stock standard solutions with micro-liter syringes of different volume 25-500 µL (Hamilton, Bonaduz). They were stored under refrigeration at about 7 °C and used for calibrations and for sample spiking in fortification experiments.

The two metabolites of pirimicarb PMC-DF and PMC-D were only available as analytical solutions. They were stored at - 20 °C and used directly or diluted when necessary. To prevent photo-degradation of pirimicarb and its two metabolites their standard solutions were all stored in brown glasses.

Solvents

Acetone, cyclohexane, ethyl acetate, hexane (85% n-hexane), methanol, dichlomethane were all of the pure grade "for residue analyses" and purchased from J. T. Baker. Acetonitrile for HPLC/UV analyses was of a quality "gradient grade" and also from J. T. Baker.

Purest water for HPLC/UV analyses was obtained with a Seralpur Pro 90 equipment (Serapur GmbH) by demineralizing water with an ion exchange cartridge, removing organic impurities with absorbent carbon and resin, and filtering particles with a membrane.

Chemicals and sorbents

aluminum oxide 90 neutral (active grade I, 0.063 - 0.200 mm)	E. Merck
anhydrous sodium sulfate (p.a.)	E. Merck
cation exchange resin AG 50-WX-80	Sigma
celite 545 (0.01 - 0.04 mm)	E. Merck
dodecane (> 99%)	E. Merck
florisil® PR, 60-100 mesh	Fluka
lithium bromide (p.a.)	E. Merck
potassium hydroxide solution (1 n)	Riedel-de Haën
potassium dihydrogen phosphate (p.a.)	E. Merck
sodium chloride (p.a.)	E. Merck
sulfuric acid (95 - 97%, p.a.)	E. Merck
tetramethylammonium hydrogensulfate	E. Merck

Reagent and materials for derivatization

Reagent: anhydrous trimethyl orthoformate (≥ 99.8%) from Aldrich Chem

Materials: thick walled 5-mL graduated reaction vials (Wheaton 986299, with 20-400 screw cap), equipped with teflon-faced silicone septa (Wheaton 240586, ϕ 20 mm)

Materials used for solid phase extraction

Baker solid phase extraction system (spe* 12G), a vacuum unity made of glass for sample preparation with bakerbond spe cartridges (J. T. Baker).

Bakerbond spe* Octadecyl (C_{18}) one way cartridges (7020-03), end capped, 3-mL cartridge with 500 mg sorbent, and bakerbond spe* quaternary amine (NR_4^+) one way cartridge (7091-03), 3-mL cartridge with 500 mg sorbent, purchased from Mallinckrodt Baker, Inc.

Materials used for laboratory lysimeter experiment

PVC columns (30 cm length, 15.2 cm inner diameter)
water penetrable microporos membrane (NY 5 HC; Hydro-Bios)
variable pipette (Stepper 411, Socorex)
membrane pump (ND100 KT.18; KHF Neuberger)
programmable process timer (PT 810 S; Alphotronic)
disposable filter holder (0.2 µm, FP 030/3, Schleicher & Schuell)

Other materials and apparatus

analytical mesh screen, mesh size 2 mm according to DIN 4188 (Retsch)
centrifuge Megafuge 1.0 (Heraeus Sepatch)
Chromafil® one way filter with PTFE-membrane (porosity 0.45 µm), type 0-45/15 organic (Macherey & Nagel)
glass wool treated with silane (E. Merck)
horizontal shaker GFL type 3020 (Gesellschaft für Labortechnik)
incubator BK-600 (Heraeus Instruments)
rotating evaporator Büchi RE 111 with Büchi B-160 vacobox and water bath (Büchi)
round filter, No. 595, ∅ 110 mm (Schleicher & Schuell)

ultrasonic bath Sonorex RK 512 S (Bandelin electric)

All glassware used were carefully washed with detergent solution and rinsed with demineralized water, then dried at 40 °C. They were rinsed with acetone directly before use.

4.2 The DFG S19 multi-residue method

The DFG S19 multi-residue method was modified and adapted for determination of pirimicarb and its metabolites pirimicarb-desmethylformamido (PMC-DF) and pirimicarb-desmethyl (PMC-D) in soils. Sample preparation and determination procedures are briefly shown in **Figure 4.1** and described in details as followed.

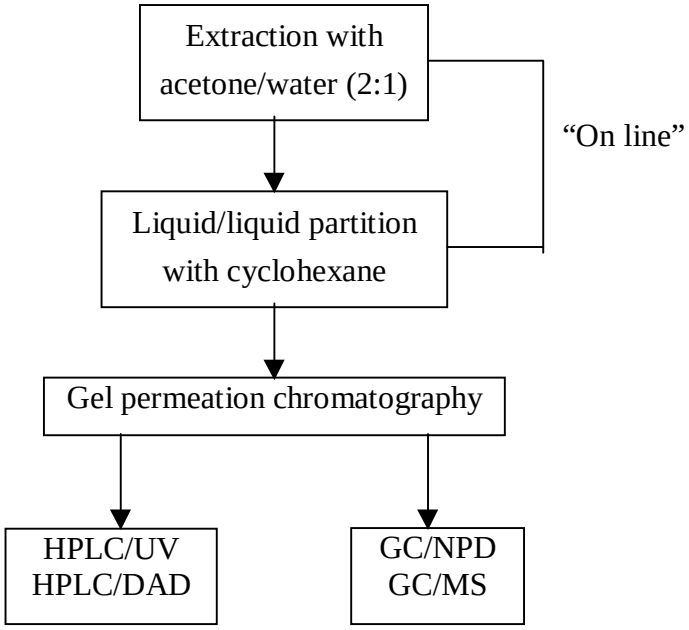


Figure 4.1 Flow chart for the analytical determination of pirimicarb, PMC-DF and PMC-D in soil (according to the DFG S19 multi-residue method).

4.2.1 Solvent extraction and partition

50 g + x g soil sample (x = native water content of the soil) was weighed into a 500-mL Erlenmeyer flask. (50 - x) mL water and 100 mL acetone were given to the sample, to achieve an acetone : water ratio of 2:1 (v/v). Then, soil sample were shaken on a horizontal shaker at 220 rev/min. Subsequently, each extraction mixture was saturated with 15 g NaCl, diluted

with 100 mL cyclohexane and shaken for another 2 h at 220 rev/min to allow the reaching of partition equilibrium. Afterwards, an aliquot of the upper organic phase was decanted into a 250-mL Erlenmeyer flask and dried over anhydrous sodium sulfate for approximately 30 min with occasional swirling. Subsequently, an aliquot of the organic phase was measured into a 500-mL round flask and successively rotary evaporated to nearly dryness. The last traces of solvent were removed with a gentle stream of nitrogen. Residues were then resolved in 5 mL cyclohexane/ethyl acetate (1:1) .

4.2.2 Clean-up by gel permeation chromatography

Clean-up steps are generally necessary for residue analysis that demands analytical methods to be sensitive enough for the determination of trace amount of substances. Therefore, an effective clean-up step with gel permeation chromatography (GPC) was employed in the DFG S19 multi-residue method and was used in this study for removing the coextractants from the soil matrix, enabling the sensitive detection of pirimicarb and especially its metabolites at concentrations lower than 50 µg/kg soil. This concentration level is required by the BBA guidelines for evaluation of pesticide analytical methods with fortification experiments (BBA, 1992).

A GPC system from the firma Abimed was used:

Pump:	Gilson pump M 305 with manometer module M 307
Injector:	Gilson injector M 231 with Gison diluter M 401 (5-mL syringe) and Rheodyne 7010 sampling loop
Injection volume:	4 to 4.5 mL (max. 4.97 mL)
Column:	38 cm × 2.5 cm I.D. with Bio Bead S-X 8 (styrol-divinylbenzol-copolymer, 8% cross-linked, 200-400 mesh) (Bio-Rad). approx. 80 g Bio Bead S-X 8 was allowed to swell in the eluent for 12 h and then filled into a glass column LC-1-23 (Latek)
Eluent:	cyclohexane/ethyl acetate (1:1)
Flow rate:	5 mL/min
Fraction collector:	Gilson M 201

After each new packing of the GPC column, fraction cutting was controlled with standard solutions of pirimicarb and its metabolites to determine the right sample fraction containing the target compounds. Before every injection, soil extracts were passed through a membrane filter to remove fine particles that might block the GPC system. An aliquot of 4 mL or 4.5 mL of a soil extract was injected and the sample fraction was collected. Then, the sample fraction was then rotary evaporated and the last traces of solvent were removed with a gentle stream of nitrogen. The residues were reconstituted in 1 mL acetonitrile for HPLC/UV and GC/MS analyses or in 1 mL ethyl acetate for GC/NPD and GC/MS analyses.

4.2.3 Fortification experiments

Fortification experiments were carried out for the assessment of the robustness, efficiency and sensitivity of the DFG S19 multi-residue method adapted for the simultaneous determination of pirimicarb and its metabolites PMC-DF and PMC-D in soils. The experiments were performed by spiking each 50 g soil (< 2 mm) in an Erlenmeyer flask with working standard solutions of pirimicarb and the two metabolites, to give fortification levels of 5, 10, 20 to 50 µg/kg soil, respectively. Working standard solutions were evenly distributed onto soil surfaces. After solvent evaporation, samples were thoroughly homogenized by shaking. Then, they were prepared using the adapted method and analyzed by HPLC/UV, GC/NPD or GC/MS to determine the recoveries and determination limits of the target compounds.

4.3 BBA method for the analysis of triallate and TCPSA

In contrast to its polar metabolite TCPSA, triallate can be determined in water and soil samples by the DFG S19 multi-residue method. For the determination of TCPSA an analytical method was briefly described in a BBA analytical method collection (BBA, 1989a). According to this method, after triallate was first extracted from water samples (BBA, 1989b), the aqueous phase was acidified by adding 2 drops of concentrated sulfuric acid and purified by shaking with 50 mL dichloromethane. The organic phase was discharged. The following partition of TCPSA into dichloromethane was based on the principle of ion pair extraction. 2 g tetramethylammonium hydrogensulfate (TBAHS) were added to the aqueous phase as phase transfer catalyst and the partition was repeated with 50 mL dichloromethane. The organic extract was dried over anhydrous sodium sulfate, concentrated on a rotary evaporator and

resolved in methanol. Subsequently, to facilitate the esterification with trimethyl orthoformate, TCPSA was transferred from its tetramethylammonium salt form to its free acid form by passing through a cation exchange resin (AG 50-WX-8, Sigma). Before GC/ECD analysis, the derivatization mixture was recommended to be cleaned up on florisil column. The clean-up conditions were, however, not given in the BBA method and were tested out in this work.

Florisil was activated overnight at 140 °C and then partially deactivated with 5 % demineralized water. 1.5 g of the freshly prepared sorbent were filled into a glass column (8 mm I.D.) and covered with a layer of anhydrous sodium sulfate. After conditioning with 15 mL of hexane, a methylated sample mixture was dissolved or suspended into hexane and quantitatively transferred into the column. The column was washed with 17 mL ethyl acetate/hexane 2:98 (v/v), before TCPSA was eluted with 17 mL ethyl acetate/hexane 10:90 (v/v). Finally, the eluate was concentrated and the residues were dissolved in hexane for GC/ECD analysis.

4.4 New method development

4.4.1 Solvent extraction of soil samples

For the determination of triallate and, especially, TCPSA, a new analytical method based on solid phase extraction (SPE) was developed in this work. At first, both target compounds should be extracted from soil, to facilitate the following enrichment of soil extracts with SPE.

100 mL methanol were used for the extraction of a 50 g soil sample. 8 g Celite 545 were added to each soil sample to accelerate the following sample filtration. Samples were first ultrasonically treated for 10 minutes and then shaken overnight on a horizontal shaker at 220 rev./min. Afterwards, samples were filtrated through a Buchner funnel. About 30 mL of methanol was additionally given to rinse each sample flask several times. The rinse was filtrated through the Buchner funnel, as well. Subsequently, supernatants were rotary evaporated to about 30 mL at 40 °C. Exact volumes of the remaining extracts were measured. To facilitate the subsequent solid phase extraction, the remaining extracts were diluted with demineralized water to yield a methanol/water ratio of 1:4 (v/v).

The solvent extraction step can be omitted for water samples. They were directly prepared by solid phase extraction or simply after a filtration when there were tiny particles present in the water samples.

4.4.2 Solid phase extraction

NR_4^+- and C_{18}-cartridges were used for the separated extraction of triallate and TCPSA from water samples or soil extracts. Triallate was extracted by a C_{18}-cartridge, whereas TCPSA was passed through the C_{18}-cartridge but enriched on a NR_4^+-cartridge. C_{18}-cartridges were also used for retaining some coextractants from water or soil matrices. This could lead to the reduction of matrix interfering during the extraction of TCPSA on NR_4^+-cartridges, as well as in the further sample derivatization step. It helped also to prevent the extraction capacity of NR_4^+-cartridges from being exhausted.

To perform the solid phase extraction NR_4^+- and C_{18}-cartridges were attached to Baker spe* 12G system and conditioned separately with 5 mL methanol, followed by 10 mL demineralized water. At first, methanol was allowed to soak into the entire sorbent bed of each extraction cartridge for several minutes before it was aspirated through the cartridges by applying slight vacuum. From this point on, the sorbent bed was kept wet and not allowed to drop dry until the sample enrichment was finished. After the solvent conditioning, the extraction cartridges were stacked together using adapters in a way that the C_{18}-cartridge is on the top and the NR_4^+-cartridge at the bottom. Samples were introduced into the cartridges through 75-mL reservoirs. The sample flow rate through the cartridges was regulated at about 100 mL/h by adjusting the vacuum applied. Sample flasks were rinsed twice with 10 mL water which were added to the reservoirs, as well.

After the sample enrichment was finished, cartridges were dried under full vacuum suction for about 30 minutes. Subsequently, C_{18}- and NR_4^+-cartridges were separately eluted. 7 mL methanol/ethyl acetate 1:1 (v/v) were used for each C_{18}-cartridge to release triallate from the cartridge and the eluates (triallate fraction**)** were collected. Each NR_4^+-cartridge was first washed with 5 mL methanol to further eliminate coextractants that had passed through the C_{18}-cartridge but were retained on NR_4^+ cartridges (methanol fraction). Since no TCPSA was detected in the methanol fractions by repeated tests, the methanol fractions were discarded.

TCPSA was then eluted from the NR_4^+-cartridge with 5 mL 0.5 % sulfuric acid/methanol (v/v) and the eluates (TCPSA fraction) were collected.

4.4.3 Derivatization of TCPSA

TCPSA fraction was rotary evaporated at 40 °C. The residue was transferred into a reaction vial and reduced to nearly dryness under a gentle stream of nitrogen at 50 °C to remove any methanol remained. Then, 1 mL of trimethyl orthoformate was added to the residue. The methylation was carried out at 95 ± 2 °C for 2.5 h. Afterwards, the reaction mixture was cooled down at ambient temperature. One drop of n-dodecane was given as "keeper" to each sample to prevent eventual volatile loss of TCPSA methyl ester and the excess reagent trimethyl orthoformate was removed from the reaction mixture under a gentle stream of nitrogen at 50 °C. Subsequently, for the quantitative capture of TCPSA methyl ester with hexane, a micro liquid-liquid partition was performed by adding 1 mL hexane and 1 mL demineralized water to each sample residue in the reaction vials. After 5 min ultrasonic treatment and subsequent waiting for phase separation, the upper hexane fraction containing the desired TCPSA methyl ester were directly transferred into GC-vials and analyzed by GC/ECD without further clean-up.

Because TCPSA methyl ester was not available as reference chemical, methylated TCPSA standard solutions were used as calibration standards for GC/ECD analyses. After methanol was removed from the TCPSA standard solutions at concentrations of 0.01, 0.05, 0.10, 0.50, 1.0, 2.5 and 5.0 µg/mL, respectively, 5 mL 0.5 % sulfuric acid/methanol were added to each standard. Then, derivatization and further treatment were carried out in the same way as for the TCPSA-fraction.

4.4.4 Purification of triallate fraction

Soil samples. The triallate fraction was concentrated by vacuum rotary evaporation at 40 °C and reduced to nearly dryness under a gentle stream of nitrogen at 50 °C. The residue was suspended in 1 mL hexane by a short time of ultrasonic treatment. Subsequently, purification on an aluminum oxide column was performed. The neutral aluminum oxide sorbent was activated at 210 °C for 5 h and then partially deactivated by adding 7 % demineralized water

and shaken at 220 rev/min for 1 h. A glass column (1.5 cm I.D.) with teflon stopcocks was used for the column chromatography. At first, a piece of glass wool was plugged at the bottom and 10 mL hexane were added. Then, 10 g freshly prepared sorbent were filled into the column and covered at the top with a layer of anhydrous sodium sulfate. After conditioning with 10 mL hexane, the triallate fraction was quantitatively transferred into the column and eluted with 25 mL hexane/ethyl acetate mixture (95:5). Finally, the collected eluate was concentrated and reduced to dryness, then reconstituted in 1 mL hexane and analyzed by GC/ECD.

Water samples. For water samples a purification of the triallate-fraction can be omitted without sacrificing the determination limit of triallate. After the addition of n-dodecane as a keeper, the triallate fraction was concentrated and reduced to dryness with a gentle stream of nitrogen. Then, 25 mL hexane were added to each sample and dried over anhydrous sodium sulfate, since there was normally trace amount of water remained even after C_{18}-cartridges were dried over 1 h under full vacuum suction before the elution. An aliquot of the dried organic phase was further concentrated and reconstituted in 1 mL hexane for GC/ECD analyses.

4.4.5 Fortification experiments and breakthrough tests

Fortification experiments were performed for controlling the efficiency and repeatability of the analytical procedure developed for the determination of triallate and its metabolite TCPSA. They were also carried out to evaluate the determination limits of the target compounds with this new method. A series of fortification experiments were carried out with demineralized water and the two soil types from the investigation sites in Nienwohlde (NW) and Sickte (SIC) (see **Table 2.1**).

Water samples. Each 400 mL demineralized water sample was spiked with triallate and TCPSA to give concentration levels of 0.05, 0.10, 0.50, 5.0 µg/L, respectively. For each concentration level 3 - 9 replicates were analyzed.

Soil samples. Each 50 g soil sample was spiked with triallate and TCPSA to give spiking levels of 5, 10, 20, 50 µg/kg soil, respectively. For each spiking level 2-7 replicates were analyzed.

To assess the breakthrough potential of TCPSA during soil extracts enrichment on NR_4^+-cartridges, breakthrough experiments were performed by using two NR_4^+-cartridges stacked together. Soil extracts were allowed to pass through the two NR_4^+-cartridges one after another. Afterwards, the two NR_4^+-cartridges were separately eluted and analyzed by GC/ECD after derivatization.

4.5 Detection methods

4.5.1 High performance liquid chromatography (HPLC)

HPLC/UV or HPLC/DAD were used for the identification and quantification of pirimicarb and its metabolites PMC-DF and PMC-D.

Apparatus: HP Series 1050 equipped with a variable wavelength UV/VIS detector or an HP 1040A diode array spectrophotometer and connected with an HP 3398A integrator (Hewlett-Packard).

Separation column: ODS Hypersil® RP-C_{18} (5 µm), 200 mm × 4.6 mm I.D. (Hewlett-Packard).

Injection volume: 20 µL

Mobile phase: A: acetonitrile; B: 0.001 M KH_2PO_4 (pH 7)

Gradient:

time [min]	A[%]	B[%]
0	20	80
6.5	33	67
14.5	45	55
15	80	20
20	80	20

Post run: 10 min

Flow rate: 1 mL/min

Detection wave length: λ = 245 nm for pirimicarb and PMC-DF, λ = 236 nm for PMC-D

4.5.2 Gas chromatography (GC)

GC/NPD

GC/NPD was employed for the quantification of pirimicarb and its metabolites PMC-DF and PMC-D.

Apparatus: HP 5890 series II with an autosampler HP 7673 (Hewlett-Packard)

Injector: split/splitless injector
splitter closed: 0.75 min
temperature: 250 °C
septum purge: 5 mL/min
split: 30 mL/min

Injection volume: 1 µL

Carrier gas: helium 4.6: 1.0 mL/min (60 °C)

Separation column: 1) DB 5 fused silica capillary column (J & W Scientific) 30 m × 0.25 mm I.D., 0.25 µm coating (95 % dimethyl - 5 % phenylpolysiloxane)
2) Stabilwax-DB (for amines and basic compounds, Rostek Corporation), 15 m × 0.25 mm I.D., 0.10 µm coating (non-bonded polyethylene glycol)

Temperature program:
1) DB 5: 100 °C (1 min) → 6 °C/min → 250 °C (15 min)
2) Stabilwax-DB: 60 °C (1 min) → 10 °C/min → 195 °C → 1 °C/min → 205 °C → 5 °C/min → 240 °C (15 min)

Detector: nitrogen-phosphorous detector (NPD)
temperature: 250 °C
hydrogen: 3 mL/min
synthetic air: 100 mL/min
nitrogen (make up gas): 30 mL/min
bead power: 580 -780 µS

GC/MSD

GC/MSD was used for the confirmation of the results from HPLC/UV or GC/NPD analysis of pirimicarb and its metabolites PMC-DF and PMC-D.

Apparatus: HP 5890 series II with an autosampler HP 7673 (Hewlett-Packard)

Injector: split/splitless injector with electronic pressure control (EPC)
splitter closed: 1 min
temperature: 250 °C

	septum purge: 5 mL/min
	split: 30 mL/min
Injection volume:	1 µL
Carrier gas:	helium 4.6: 1.0 mL/min (constant flow, vacuum condensation)
Separation column:	DB 5 fused silica capillary column (J & W Scientific)
	30 m × 0.25 mm I.D., 0.25 µm coating (95 % dimethyl - 5 % phenylpolysiloxane)
Temperature program:	
	100 °C (1 min) → 6 °C/min → 250 °C (15 min)
Detector:	mass selective detector HP 5970B (Hewlett-Packard)
	transfer line: 280 °C, directly coupled
	ionization: electron impact ionization (EI), 70 eV
	measurement mode: selected ion monitoring (SIM):
	fragment ions for quantification: pirimicarb: m/z 166
	PMC-DF: m/z 72
	PMC-D: m/z 152

GC/MS (SSQ)

GC/MS (SSQ) was alternatively applied for the confirmation and quantification of pirimicarb and its metabolites PMC-DF and PMC-D. It was also used for the proof of the successful methylation of TCPSA, and served further as a confirmation measurement to the results of GC/ECD determination of triallate and TCPSA.

Apparatus:	Varian 3400 (Varian, Darmstadt) with an autosampler HP 7673 (Hewlett-Packard)
Injector:	temperature programmable injection system KAS 3 (Gerstel)
	temperature program: 100 °C (3 s) → 12 °C/s → 350 °C (300 s)
	splitter closed: 1 min
Injection volume:	1 µL
Carrier gas:	helium 4.6: 1 mL/min (60 °C)
Separation column:	DB 1 fused silica capillary column (J & W Scientific)
	15 m × 0.25 mm I.D., 0.10 µm dimethyl coating
Temperature program:	

60 °C (1 min) → 10 °C/min → 280 °C (7 min)

Detector: quadrupole mass spectrometer SSQ 70 (Finnigan MAT)

transfer line: 280 °C, directly coupled

ionization: electron impact ionization (EI), 70 eV

negative chemical ionization (NCI),

reactant gas: methane (source pressure 66.5 Pa at 120 °C)

measurement mode: 1) full scan: 60 - 300 amu

2) selected ion monitoring (SIM):

fragment ions for quantification: pirimicarb: m/z 166

PMC-DF: m/z 72

PMC-D: m/z 152

GC/ECD

GC/ECD was applied for the quantification of triallate and its metabolite TCPSA in the form of its methyl ester.

Apparatus: HP 5890 series II with an autosampler HP 7673 (Hewlett-Packard)

Injector: split/splitless injector

splitter closed: 0.75 min

temperature: 280 °C

Injection volume: 1 µL

Separation column: DB 5 fused silica capillary column (J & W Scientific, Folsom)

30 m × 0.25 mm I.D., 0.25 µm coating (95 % dimethyl - 5 % phenylpolysiloxane)

Temperature program:

triallate: 60 °C (3 min) → 20 °C/min → 160 °C → 4 °C/min → 210 °C → 20 °C/min → 280 °C (15 min)

TCPSA: 60 °C (3 min) → 10 °C/min → 140 °C → 2 °C/min → 170 °C → 20 °C/min → 280 °C (15 min)

Carrier gas: helium 4.6: 1.0 mL/min (60 °C)

Septum purge: 5.5 mL/min

Split: 45 mL/min

Make up gas (nitrogen): 50 mL/min

Confirmation column:	DB608 capillary column (J & W Scientific, Folsom)
	30 m × 0.539 mm I.D., 0.83 µm coating (specially prepared cyanopropylphenyl methyl silicone)
Temperature program:	
triallate:	60 °C (3 min) → 20 °C/min → 190 °C → 4 °C/min →
	220 °C → 20 °C/min → 280 °C (15 min)
TCPSA:	60 °C (3 min) → 10 °C/min → 170 °C →
	2 °C/min → 190 °C → 20 °C/min → 280 °C (15 min)
Carry gas:	helium 4.6: 5.8 mL/min (60 °C)
Septum purge:	5.4 mL/min
Split:	43 mL/min
Make-up gas (nitrogen):	56 mL/min
Detector:	^{63}Ni electron capture detector (ECD)
	temperature: 330 °C

4.5.3 Identification and quantification

Pirimicarb and its metabolites PMC-DF and PMC-D

The formation of PMC-DF and PMC-D as degradation products of pirimicarb in soil samples from laboratory batch and field experiments was verified by GC/MS screening in full scan mode. Quantification of pirimicarb and the two metabolites in the HPLC/UV, GC/NPD and GC/MS analyses was carried out using the external standard calibration technique. Calibration curves were recorded with analytical standard solutions or matrix standards in the concentrations range of 0.125 ng/µL to 10 ng/µL for pirimicarb and 0.125 ng/µL to 5 ng/µL for the metabolites. To control the repeatability of peak areas, periodic recalibration with standard solutions after every 10-15 sample injections was performed throughout all the analyses. The quantification results were confirmed using two different determination methods or using two separation columns of different polarities.

Triallate and its metabolite TCPSA

To prove that TCPSA was successfully converted to its methyl ester through methylation with trimethyl orthoformate, GC/MS analyses with EI and NCI detection in full scan mode was performed.

The identification of TCPSA as triallate degradation products in soil was realized by comparing the retention times and mass spectra of the target compound found in soil or water samples with that of methylated TCPSA standard using GC/MS or by comparing the retention times using GC/ECD with two capillary columns of different polarities (DB 5 and DB 608).

The quantification of triallate and TCPSA methyl ester was carried out using the external standard calibration technique. For the routine analyses by GC/ECD, a DB 5 capillary column was used and the quantification results were confirmed with a DB 608 capillary column. Separation conditions were described in **Chapter 4.5.2**. Calibration curves in the concentration range of 0.1 ng/µL to 2.5 ng/µL as well as in the concentration range of 0.01 ng/µL to 0.5 ng/µL were recorded using diluted triallate working standard solutions and methylated TCPSA standard solutions. The separated calibrations in two linear dynamic ranges were necessary for an accurate quantification, especially for the quantification of trace amounts of triallate and TCPSA near the determination limits.

4.6 Laboratory batch experiments

Laboratory batch experiments were carried out under controlled experimental conditions to investigate the degradation of pirimicarb and triallate in different soil types, in especial, with respect to the determination of their corresponding metabolites. Information obtained in these experiments are useful for the general comparison of degradation behavior between different pesticides and for a preliminary estimation of their possible fate in the real application situations.

The experiments were carried out according to the BBA guideline for pesticide assessment in the registration procedures (Schinkel et al., 1986). Fresh soils were scattered sampled from the superficial 0-5 cm soil layers at the investigation sites. Then, single soil samples were mixed thoroughly and sieved through an analytical mesh screen (< 2 mm) to prepare

representative mixed soil samples. Then, soil moisture was determined according to DIN 38414, part 2 (DIN, 1985). To avoid the loss of microbial activities in fresh prepared soil samples, degradation experiments were started within 7 days after the soil sampling from the fields. In addition, soil moisture was maintained in laboratory batch experiments by compensation of the evaporated water with demineralized water.

For each sample, a certain amount of soil (equivalent to 50 g dry soil) was weighed into a 200-mL Erlenmeyer flask and spiked with 1 mL of pirimicarb or triallate methanol solution to give a definited initial concentrations of each active substance. The spiked soil samples were thoroughly shaken to let the active substances to be well-distributed in the soil samples. Then, by adding demineralized water soil moisture was adjusted to 40 % of the maximum water holding capacity of the soil. Subsequently, flasks were covered with parafilm with several small holes on the film surface for a free air exchange. Soil samples were incubated at 24 ± 1 °C in the dark. By compensation of the evaporated water with demineralized water every 3 to 4 days, the soil moisture was remained nearly constant throughout the incubation period. Afterwards, soil samples were stored at - 20 °C until analysis.

Two laboratory batch experiments were carried out to study pirimicarb degradation in the clayey silt soil from Neuenkirchen (NK-soil) and in the silty sand soil from Nienwohlde (NW-soil), respectively. The spiking level of pirimicarb was 1150 µg/kg for NK-soil and 1010 µg/kg for NW-soil, equivalent to 1150 and 1010 g A.S./ha, respectively. These spiking levels are about two- to fivefold of the normal application rates. The soil incubation periods were 2 h, 1, 3, 7, 14, 21, 28 (29), 42 (43) and 56 (57) days for NK-soil (or NW-soil).

Two labotory batch experiments were carried out to study triallate degradation in the loamy sand soil from Sickte (SIC-soil). The spiking level of triallate was 4000 µg/kg soil or 4000 g A.S./ha, about the tripled application rate, for one batch experiment; and 2600 µg/kg soil or 2600 g/ha, the doubled application rate for another one. The soil incubation periods were 2 h, 7, 14, 21, 28, 35, 53 days, and 2h, 3, 7, 21, 28, 43, 59 days, respectively. After incubation, soil samples were stored at -20 °C until analysis.

The higher initial concentrations of pirimicarb and triallate were chosen in order to detect the formation of the corresponding metabolites which are usually expected to appear at very low concentrations.

4.7 Field experiments

Field experiments with pirimicarb foliar application to sugar beets and direct soil application were carried out at the investigation site Neuenkirchen. According to the usual agricultural practice, 600 g Pirimor® (300 g A.S. pirimicarb) solved in 400 L water were sprayed to a plot of approximate 0.35 ha in the sugar beet field in Neuenkirchen, corresponding to an application rate of 857 g/ha. Pirimor® preparation was sprayed to two areas, one was covered with sugar beets, the other was bare. Then, soil samplings were performed 1 h as well as 1, 3, 7, 13, 20, 27, 41 and 55 days after the application. Soil samples were also taken from another sugar beet plot 13, 26 and 54 days after the pirimicarb foliar application on June 14, 1995. The application rate was the same as that sprayed on June 27, 1995.

A field experiment with pirimicarb foliar application to sugar beets was also carried out at the investigation site in Nienwohlde, with the same application rate as that used in Neuenkirchen. Pirimor® preparation was sprayed to the sugar beet plot on June 20, 1995. Soil samplings were performed 1 h as well as 1, 3, 7, 14, 21, 28, 42 and 56 days after the application.

At both investigation sites, soil samples were taken with a bulb planter from the superficial soil layers 0-5 cm and 5-10 cm. 12 single samples were taken scattered from the whole applied area and mixed up to give one mixed sample. All mixed samples were field fresh sieved through an analytical mesh screen (< 2 mm) and stored at - 20 °C until analysis. In the same way, a mixed sample was taken at every sampling time from the neighboring sugar beet field having no pirimor® application and used as the blank sample.

4.8 Sorption equilibrium studies

The distribution coefficient of a substance between soil and soil solution is an important parameter for characterizing the adsorption-desorption processes as well as for estimating its

leaching potential into deeper soil layers. Using the method described by Stalder & Pestemer (1980) and Bunte (1991), the soil/water distribution coefficients (K_d values) of pirimicarb and its metabolites PMC-DF and PMC-D in NK-soil and NW-soil were determined in sorption equilibrium studies, so as the K_d values of triallate and its metabolite TCPSA in SIC-soil.

To determine K_d values of pirimicarb, 500 µL pirimicarb standard solution at a concentration of 100 ng/µL were pipetted into an Erlenmeyer flask and the solvent was carefully evaporated with a gentle stream of nitrogen. After the adding of 10 mL demineralized water and 10 min treating in an ultrasonic bath, 50 g air-dried soil were added to the flask and mixed thoroughly with the aqueous solution, giving an initial concentration of 50 µg pirimicarb/50 g soil. Samples were then left to stand for 24 h at 7 °C to reach the distribution equilibrium between soil and water. Subsequently, 60 mL demineralized water were added to each sample and the samples were shaken for 1 h on a horizontal shaker at 220 rev/min. Then, samples were centrifuged at 4000 rev/min for 30 min. Supernatants were decanted and 40 mL aliquots were further prepared, in which 100 mL acetone, 100 mL cyclohexane and 15 g sodium chloride were added and the mixture was shaken on a horizontal shaker at 220 rev/min for 2 h. Then, the organic phase was collected and dried over anhydrous sodium sulfate. An aliquot of the organic phase was rotary evaporated to about 1 mL and the last traces of solvent were removed with a gentle stream of nitrogen. The residue was resolved in 1 mL ethyl acetate and analyzed by GC/NPD or GC/MS. The determination was carried out with four replicates for each soil type.

To reduce the usage of the expensive reference chemicals of PMC-DF and PMC-D, their K_d values were determined with reduced sample size, namely a starting concentration of 10 µg metabolite/10 g soil. The volume of solvents used in the further sample preparation was reduced accordingly. The determination was carried out in duplicates for each metabolite and each soil type.

The soil/water distribution coefficients (K_d values) of triallate and TCPSA in SIC-soil were determined in the similar way. The initial concentrations were 66 µg/50 g soil for triallate and 5 µg/50 g soil for TCPSA. The supernatants (soil solutions) obtained were further prepared as that described for water samples (see **Chapter 4.4**). The determination was carried out with four replicates for each target compound.

The soil/water distribution coefficient (K_d value) was calculated as the ratio of the concentration adsorbed on soil to the concentration in the soil solution as shown in the following equation:

$$K_d = \frac{(M_T - M_L)}{M_L} \frac{M_{H_2O}}{M_{Soil}}$$

where M_T and M_L are the total amount of the analyte originally added and the amount determined in the soil solution; M_{H_2O} and M_{Soil} are the amounts of water and soil.

4.9 Laboratory lysimeter experiments

Laboratory lysimeter experiments with undisturbed soil monoliths were carried out to simultaneously investigate the leaching tendency of triallate and TCPSA through simulating real hydraulic conditions in the field.

4.9.1 Sampling and preparation of soil monoliths

Soil monoliths whose properties were listed in **Table 2.1**., were sampled at the investigation site of BBA, according to the sampling technique described by Nordmeyer and Aderhold (1995, 1994). The technical equipment is shown in **Figure 4.2**.

PVC columns (30 cm length, 15.2 cm I.D.) were vertically put onto the blank soil surface and hydraulically pushed into the soil to a 30 cm depth. Then, these lysimeter columns filled with undisturbed monoliths (approximately 10 kg soil with 14 % average soil moisture) were dug out of the soil and transported into the lysimeter station. A cover equipped with a water penetrable microporos membrane (NY 5 HC; Hydro-Bios) for percolate draining was fitted onto the bottom of each lysimeter column and sealed up with silicone sealant. Subsequently, soil monoliths were saturated with water through capillary effect, in which water was allowed to penetrate slowly into the soil monolith from the bottom within 2 days. In this way the natural soil structure was not disturbed during the saturation. Afterwards, soil monoliths were allowed to drain for another two days, so that the soil moisture reached its maximum field holding capacity. Then, the draining cover was connected to a vacuum box. In order to

simulate real hydraulic conditions and to prevent the occurrence of stagnant water, a suction power of -10 hPa was applied which ensured continuous water downward movement through the soil monoliths.

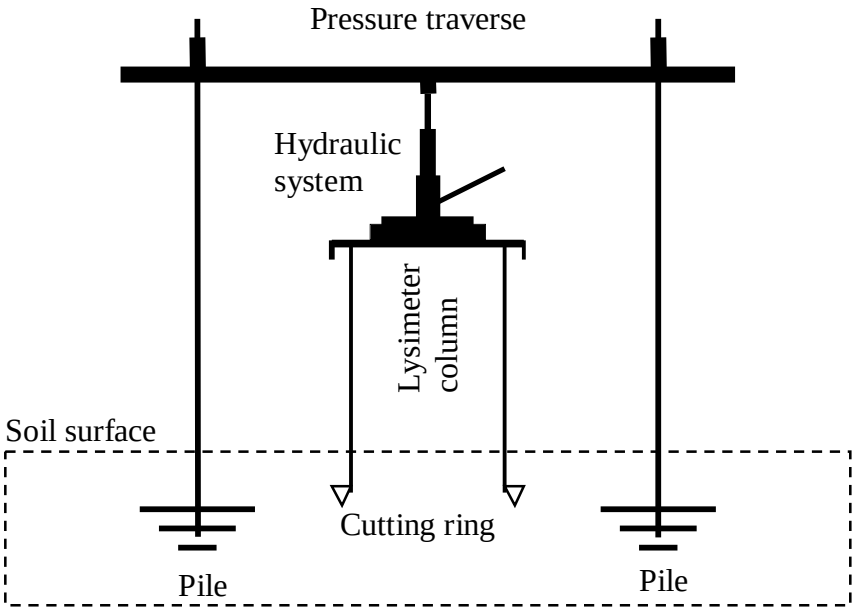


Figure 4.2 Hydraulic sampling system for undisturbed soil monoliths (Nordmeyer and Aderhold, 1994)

4.9.2 Application of triallate and TCPSA

In two lysimeter experiments (lysimeter I and II), 5200 µg triallate were directly soil-applied as Avadex BW® solved in 10 mL demineralized water. This application rate (2.89 kg triallate/ha) was approximately the doubled amount usually applied in agricultural practice (1.2 - 1.7 kg A.S./ha suspended in 400 L water/ha), so as to ensure the determination of triallate residue and TCPSA formed in percolates and soil samples.

To achieve an uniform distribution of analytes on the soil surface, a wire mesh with 40 cells of 2 x 2 cm^2 was put on the column and 5 x 50 µL of the solution were given into each cell using a variable pipette (Stepper 411; Socorex). To minimize losses via volatilization, triallate was subsequently incorporated into the superficial soil layers.

In case the amount of TCPSA formed from triallate degradation was too low to be determined in percolates, in another lysimeter experiment (lysimeter III) 5000 µg TCPSA dissolved in 10 mL demineralized water were applied according to the application rate of triallate, so as to ensure the comparable investigation on the mobility of triallate and TCPSA in soil.

Lithium bromide was additionally used as a conservative tracer to control water movement by the determination of breakthrough curves for each lysimeter. The application amount was equivalent to 36 kg bromide/ha.

4.9.3 Incubation and sprinkler irrigation of soil monoliths

Directly after the application, lysimeter I and III were installed in the lysimeter experimental station as illustrated in **Figure 4.3** (Nordmeyer and Aderhold, 1995).

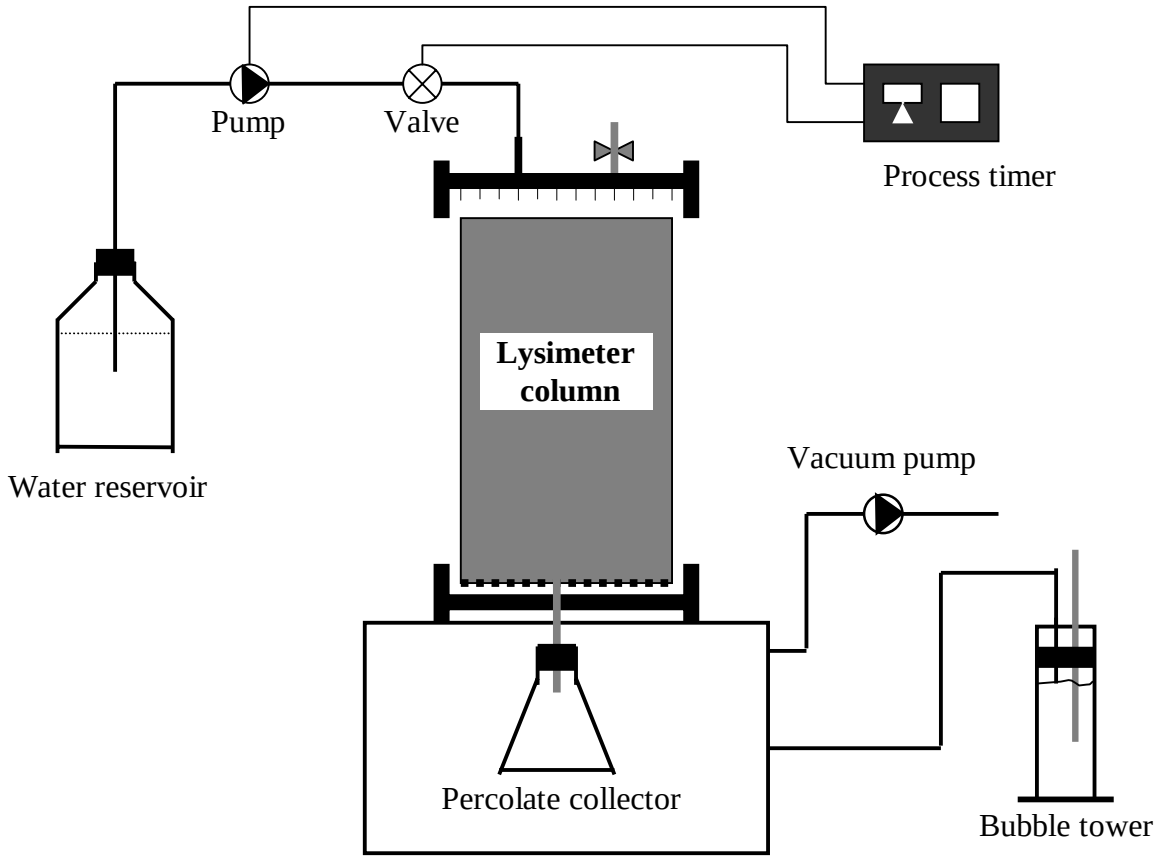


Figure 4.3 Lysimeter experimental station (Nordmeyer and Aderhold, 1995)

The leaching behavior of triallate and TCPSA which were applied to these two lysimeter, separately, as well as the formation of TCPSA as degradation product of triallate in lysimeter I were investigated. Experiments were carried out at 10 °C, taking into consideration of an average soil temperature during triallate application in spring.

For comparison, lysimeter II was preliminary incubated at 20 °C in the dark for 14 days to enhance microbial degradation of triallate and the formation of TCPSA. Then, it was further treated like lysimeter I and III. This approach was supplemented by a simultaneous laboratory batch experiment of triallate degradation to investigate the formation of TCPSA under such conditions. Fresh soil samples, with 12 % water content and 20 ± 2 mg biomass-C/100 g dry soil which was determined on a Sapromat apparatus according to the substrate induced respiration (SIR) method described by Beck et al. (1984), were taken from the superficial 0-5 cm soil layers and spiked with Avadex BW® at a level of 2,88 mg/kg soil. Incubation temperature was 20 °C within the first 14 days and then maintained at 10 °C until the end of the incubation period. Soil samples were taken 2 h, 7, 14, 28, 45, and 56 days after application.

Irrigation of the soil monoliths was performed using a sprinkler system. The sprinkler head (16 x 16 cm^2) with 96 needles ensured an uniform distribution of irrigation water over the soil surfaces. It was connected to a water reservoir through a magnetic valve and a membrane pump (ND100 KT.18; KHF Neuberger). Irrigation intensity and time, controlled by a programmable process timer (PT 810 S; Alphotronic) were 5 mm/h for 2 h and twice in 7 days with a 24 h interval between the first and the second irrigation. This corresponded to a weekly precipitation of 20 mm. In lysimeter I and III, first irrigation started 3 h after the application of triallate or TCPSA, while lysimeter II was irrigated first after the 14 days incubation period. Totally, precipitation events with 140 mm and 100 mm, respectively, were simulated within 45 days. The -10 hPa suction power was applied during the irrigation as well as after the irrigation through the percolates collection time to prevent stagnant water in the soil monolithes.

4.9.4 Sampling and analysis of percolates and soils

Percolates were sampled for each irrigation cycle in 250-mL glass flasks. They were centrifuged at 4000 rev/min for 15 min and microfiltered to remove the particulate matters

released out of the lysimeter columns by infiltration water. The samples were then prepared immediately according to the method developed and analyzed by GC/ECD. Bromide in the percolates was determined by ion chromatography (690 Ion Chromatograph; Metrohm) using the external standard calibration technique. Calibration curves were recorded with standard solutions of lithium bromide in the concentrations range of 1-10 µg/mL. When necessary, percolate samples were diluted with demineralized water, before they were injected with an autosampler into the ion chromatographic system.
At the end of lysimeter experiments, soil samples from different soil depths (0-5, 5-10, 10-20 and 20-30 cm) were taken with a spatula from each soil monolith. Then, they were separately homogenized, weighed, and soil inherent water contents were determined. Aliquots of the soil samples were frozen at -20 °C until analysis.

To compare with the methanol extraction of soil samples used in the new method, extraction of soil samples with demineralized water was also carried out. According to the method for soil/water distribution coefficient (K_d value) determination that was described in **Chapter 4.8**, a definite amount of water was added to soil samples to give a water/soil-ratio of 70:50. The samples were shaken overnight and centrifuged. Aliquots of supernatants were further prepared and analyzed.

4.10 Waste disposal and recycling

Organic solvent wastes from sample preparation in the residue analysis as well as remaining standard solutions were generally collected and brought to the combustion. The recycling of the solvent mixture cyclohexane/ethyl acetate 1:1 (v/v) was an exception. The waste of this solvent mixture came from rinse, pre- and post-run fractions of the GPC, as well as from the vacuum evaporation of GPC fractions containing the target compounds. They were separately collected and recycled through rectification. The recovered azeotrope cyclohexane/ethyl acetate 44:56 (v/v) was adjusted to the volume ratio of 1:1 by adding the required volume of cyclohexane. It can be, therefore, used again for the GPC in residue analysis (Bittner et al., 1993). Acetone used for the rinse of glassware was also separately collected and applied as “technical” grade solvent for general rinse purpose.

Because of the human and ecological toxicity of dichloromethane, it was replaced by organic solvent of less toxicity as far as possible. Special attention was paid to the usage of

dichloromethane, so that no trace of it came into the sewage system (Gunschera et al., 1992). This was controlled by filling the cooling tube with liquid nitrogen during evaporation of this solvent. Condensed dichloromethane was then treated like other organic solvent wastes. Aqueous wastes from the liquid-liquid partition were stripped with nitrogen stream under the fume cupboard, before they were discharged into the sewage system.

Solid wastes in this work included extracted soils (containing sodium chloride or celite), used sodium sulfate and aluminum oxide. After the residual solvents in these wastes were vented under fume cupboard, they were disposed together with normal household wastes. Not-used soil samples as well as soil fractions > 2mm were brought back to the corresponding investigation sites.

5 Results and discussions

5.1 Pirimicarb and its metabolites

5.1.1 Determination of pirimicarb and its metabolites in soil

Soil sample preparation

With the DFG S19 multi-residue method (DFG, 1991) a wide variety of lipid- as well as water-soluble pesticides and metabolites can be extracted from different kinds of environmental matrices such as plant tissues, soils and sediments. It was proven in this work that pirimicarb and its metabolites PMC-DF and PMC-D could be successfully determined in soil samples with this method. Inherent water contents of samples were taken into account so that the acetone/water ratio remained constant at 2:1 (v/v) for extraction. This is of great importance for a multi-residue method to standardize the extraction conditions for samples of different water contents. The addition of water is important for a better moistening of sample materials with acetone and therefore for a more efficient extraction of pesticide residues from sample matrices. It is especially significant for the simultaneous determination of pesticides and their normally more polar metabolites. The solvent mixture of 200 mL acetone with 100 mL water used in the DFG S19 multi-residue method was reduced to 100 mL acetone with 50 mL water that has been proven to be enough for the extraction of pirimicarb and its metabolites PMC-DF and PMC-D in 50 g soil samples. Additionally, less solvent consumption can save the later solvent evaporation time and reduce the risk of volatile losses of target compounds from the soil extracts.

The subsequent liquid-liquid partition step was performed to force the extracted residues into an organic solvent which is non-miscible with water and has a relative low-boiling point. This enabled easier vacuum solvent evaporation and avoided losses of volatile compounds from the extracted residues. Because of its middle polarity in the eluotropic series and its low boiling point, dichloromethane was used in the DFG S 19 multi-residue method to extract a variety of compounds of different polarity from the aqueous phase. However, with increasing concern about the human and ecological toxicity of dichloromethane, efforts have been made to replace dichloromethane with organic solvents of less environmental pollution (Koinecke,

1994). Therefore, in stead of dichloromethane cyclohexane was used in this work, and comparable recoveries were obtained for pirimicarb and its two metabolites by partition either with dichloromethane or with cyclohexane in fortification experiments. The partition was carried out "on-line" according to the method described by Steinwandter (1989) to save the extracts filtration step. Extraction mixtures were saturated with sodium chloride to reduce the solubility of relatively polar compounds in water so that a quantitative transfer of residues into the organic phase and a better phase separation could be achieved.

After the vacuum solvent evaporation residues were further cleaned up by gel permeation chromatography (GPC). GPC is an universal applicable clean-up method widely used in residue analysis for eliminating some components from sample matrices that may interfere the target compounds analysis. Compounds, whether they are polar, nonpolar or thermolabile, can be separated according to their molecular sizes, without decomposition or irreversible adsorption in the GPC column. The separation is based on the principle of size exclusion chromatography (Thier and Frehse, 1986). Since soil organic matters consists, to a great extend, of large humic substances, whereas pesticides and their metabolites are comparably small molecules, GPC is the proper choice for the primary clean-up of soil extracts. Through fraction cutting of GPC eluates, many coextractants can be eliminated. Additional column clean-up methods with sorbents such as Florisil, aluminum oxide, or silica gel are sometimes used for further elimination of interfering coextractants.

There are only one functional group difference in the molecular structures of pirimicarb and its metabolites PMC-DF and PMC-D. So it is to expect that these three target compounds could be eluted together from the GPC-column within a relatively narrow fraction. Fraction cutting with mixed standard solutions of pirimicarb, PMC-DF and PMC-D proved that they all appeared within the fraction of 120-190 mL under the experimental conditions described in **Chapter 4.2.3**. Without any further clean-up steps, sensitive and relatively non-interfered determinations by HPLC/UV, GC/NPD or GC/MS were achieved for all three compounds in the samples of the two different soil types used.

Sample analysis

Pirimicarb and the two metabolites PMC-DF and PMC-D could be analyzed either by high performance liquid chromatography with UV or diode-array spectrometric detection

(HPLC/UV or HPLC/DAD) or by gas chromatography with specific nitrogen-phosphorous detector or mass spectrometer (GC/NPD or GC/MS). In this work both HPLC and GC determination methods were developed for the simultaneous determination of pirimicarb and the two metabolites.

Generally, capillary gas chromatography has a higher separation capacity than that of high performance liquid chromatography. However, HPLC shows advantages for analyzing more polar substances which needed to be transferred into volatile and stable derivatives to facilitate GC analysis. To use HPLC/UV or HPLC/DAD for the determination of pesticides and their metabolites in samples of complex matrices like soil samples, the analytes should possess specific chromophores that give characteristic UV-spectra with absorption maximum clearly over the transparency minimum of solvent eluents used ($\lambda > 200$ nm). Otherwise chromatographic separations would be impossible because of matrix interference that could not be totally eliminated by even comprehensive clean-ups. The UV-spectra show absorption maximum of $\lambda = 245$ nm for pirimicarb and PMC-DF and $\lambda = 236$ nm for PMC-D. Therefore, all three compounds in soil extracts could be analyzed by HPLC/UV. The analytical parameters described in **Chapter 4.5.1** were chosen by using standard solutions as well as matrix standards. Two chromatograms of a standard solution and a matrix standard, respectively, are depicted in **Figure 5.1**. It was found by fortification experiments that external calibrations using matrix standards was more suitable than the standard solutions for the quantification of HPLC analytical results. It seems that matrix influences could be compensated to a certain degree by using matrix standards. To get matrix standards, blank soil samples were prepared with the sample preparation method discussed in **Chapter 4.2**. Then, standard solutions of the target compounds were added to the blank soil extracts to give matrix standards in a concentration range of 0.1 - 5.0 µg/L for the two metabolites and 0.1 - 10.0 µg/l for pirimicarb. Linear responses of peak areas or peak heights to the analytes concentrations were obtained in these concentration ranges with correlation coefficients $r \geq 0.99 - 0.999$.

A second approach was to determine pirimicarb and its metabolites PMC-DF and PMC-D by using GC/NPD, because there are 4 nitrogen atoms in each of their molecules that promise sensitive detection with the selective nitrogen-phosphorous detector. Unlike N-methyl carbamates, which are characterized as too thermolabile to be directly analyzed by the gas chromatography (Goewie and Hogendoorn, 1987; Wüest and Meier, 1983; Fogy et al., 1979

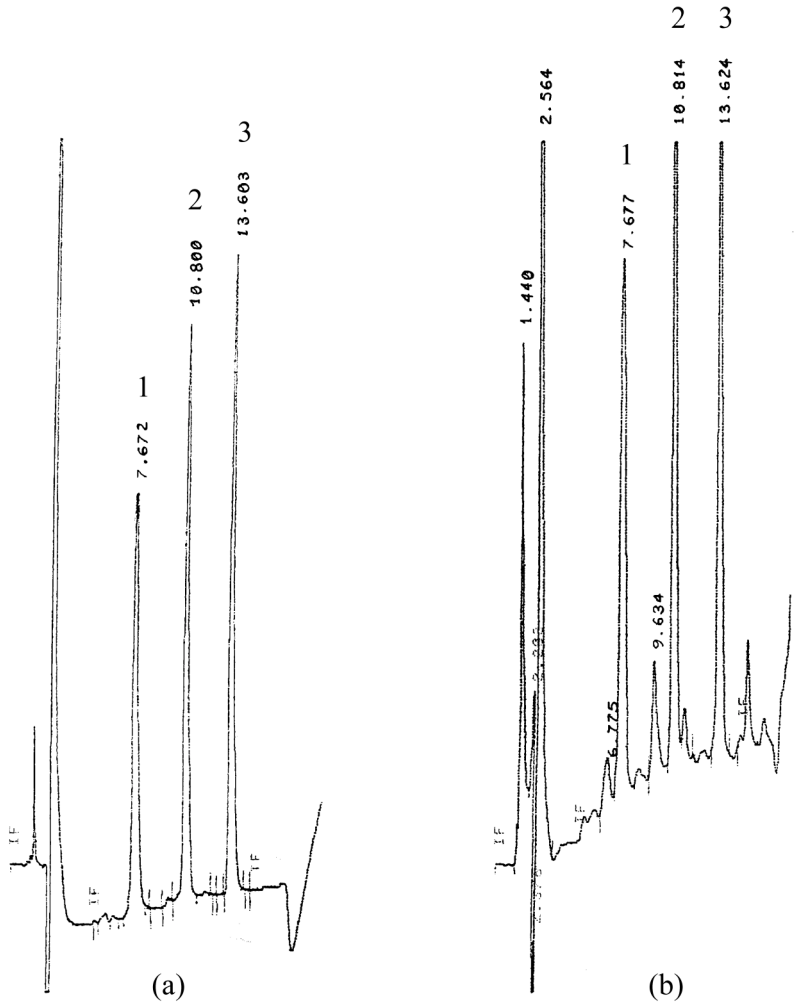


Figure 5.1 HPLC/UV chromatograms of (a) a standard solution and (b) a matrix standard of PMC-D (1), PMC-DF (2) and pirimicarb (3) at a concentration of 1.0 ng/µL.

& 1980), no decomposition of the N,N-dimethyl carbamate pirimicarb was found up to an injection temperature of 300 °C and on-column injection was not necessary (Wüest and Meier, 1983). Therefore, pirimicarb can be routinely analyzed by GC/NPD. It has also been reported that PMC-DF and PMC-D could be determined without derivatization with pirimicarb and other carbamates by GC/NPD using a well deactivated non-polar separation column such as DB-1 (Brauckhoff and Thier, 1987). It was found in this work that PMC-DF was well separated from pirimicarb and PMC-D, but a base-line separation of pirimicarb and PMC-D was not achievable by GC/MS analysis with a DB-1 under the analytical conditions described in **Chapter 4.5.2**, as shown in a reconstructed ion current chromatogram presented in **Figure 5.2**. Changing temperature programs had no obvious effect on the chromatographic performance of the DB-1. Thus, the DB-1 was only applied by the GC/MS fragmentography,

where different SIM masses were chosen for the quantification, namely m/z 166 for pirimicarb and m/z 152 for PMC-D.

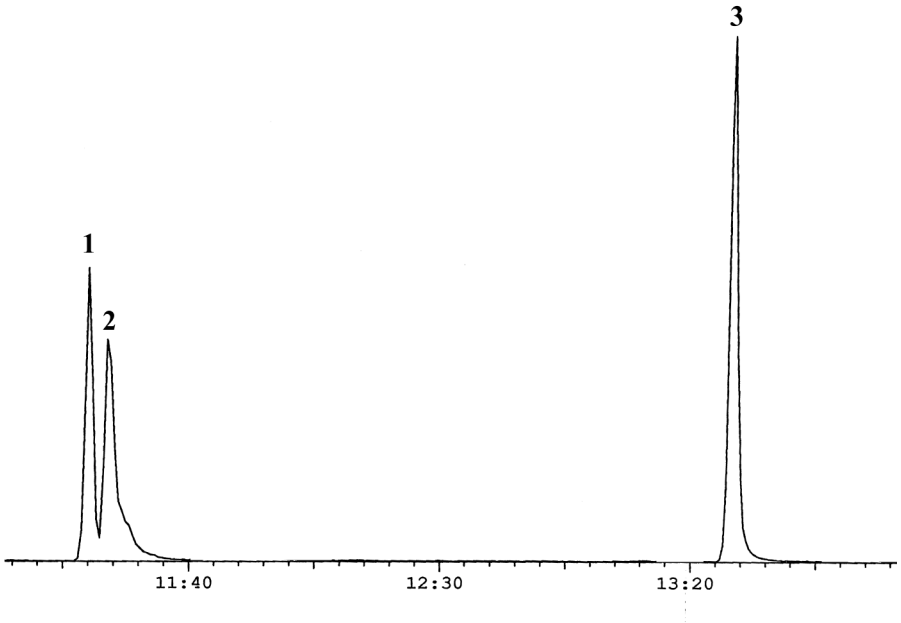


Figure 5.2 Reconstructed ion current chromatogram of a standard solution of Pirimicarb (1), PMC-D (2) and PMC-DF (3) at a concentration of 5 ng/µL by GC/MS analysis with DB-1 capillary column.

The DB-5 was found to be a proper separation column for the separation of the three target compounds by GC/NPD analysis. With this method, pirimicarb and PMC-D were base-line separated from each other (**Figure 5.3**).

As a consequence of the selectivity of nitrogen-phosphorous detector (NPD) and in comparison to the rather universal UV-detector, less signals of the sample matrices emerged in the gas chromatograms. This means less interference from the soil matrices, showing an advantage for the determination of pirimicarb and, especially, its metabolites PMC-DF and PMC-D at very low concentrations. The responses of the three target compounds in GC/NPD analysis were comparable to those in HPLC/UV and GC/MS analyses. On the other hand, NPD is generally recognized to be relatively less stable and less reproducible. Continuous monitoring of the system is imperative to ensure optimum performance for quantitative results. Short term variations were overcome with a re-calibration process, in which standard solutions were injected for re-calibration after the injections of 10 - 15 soil extracts.

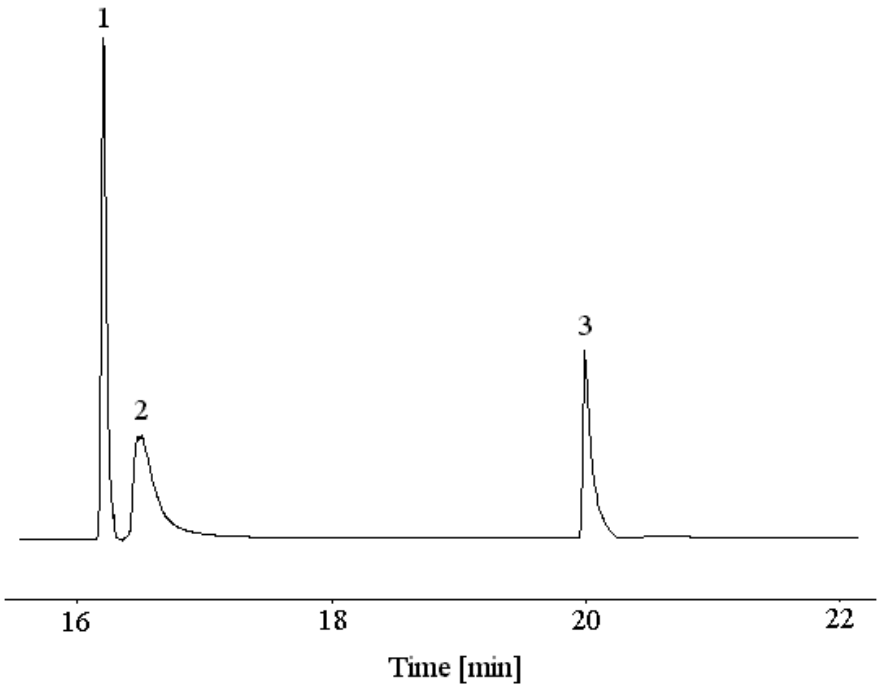


Figure 5.3 Chromatogram of a standard solution of Pirimicarb (1), PMC-D (2) and PMC-DF (3) at a concentration of 5 ng/µL by GC/NPD analysis with DB-5 capillary column.

Efforts had also been made to separate pirimicarb and PMC-D based on their expected different alkalinity, since pirimicarb is a tertiary amine and PMC-D a secondary amine. As a result, a special separation column for amines and basic compounds, the Stabilwax-DB, was successfully tested to be the most suitable separation column for the separation of the three target compounds. Therefore, the Stabilwax-DB capillary column was routinely applied in the GC/NPD analysis of pirimicarb and the two metabolites. **Figure 5.4** shows two chromatograms of a standard solution and a soil sample extract from the field experiment at Nienwohlde. Pirimicarb and PMC-DF show excellent sharp peaks, whereas the peak of PMC-D is broadened as the result of its polarity as a secondary amine.

Similar quantitative results were obtained by GC/NPD analysis with the Stabilwax-DB and the DB-5 capillary columns, as shown by the results of the field experiments at Nienwohlde presented in **Figure 5.5**. Some verifications were also performed by GC/MSD or HPLC/UV analysis.

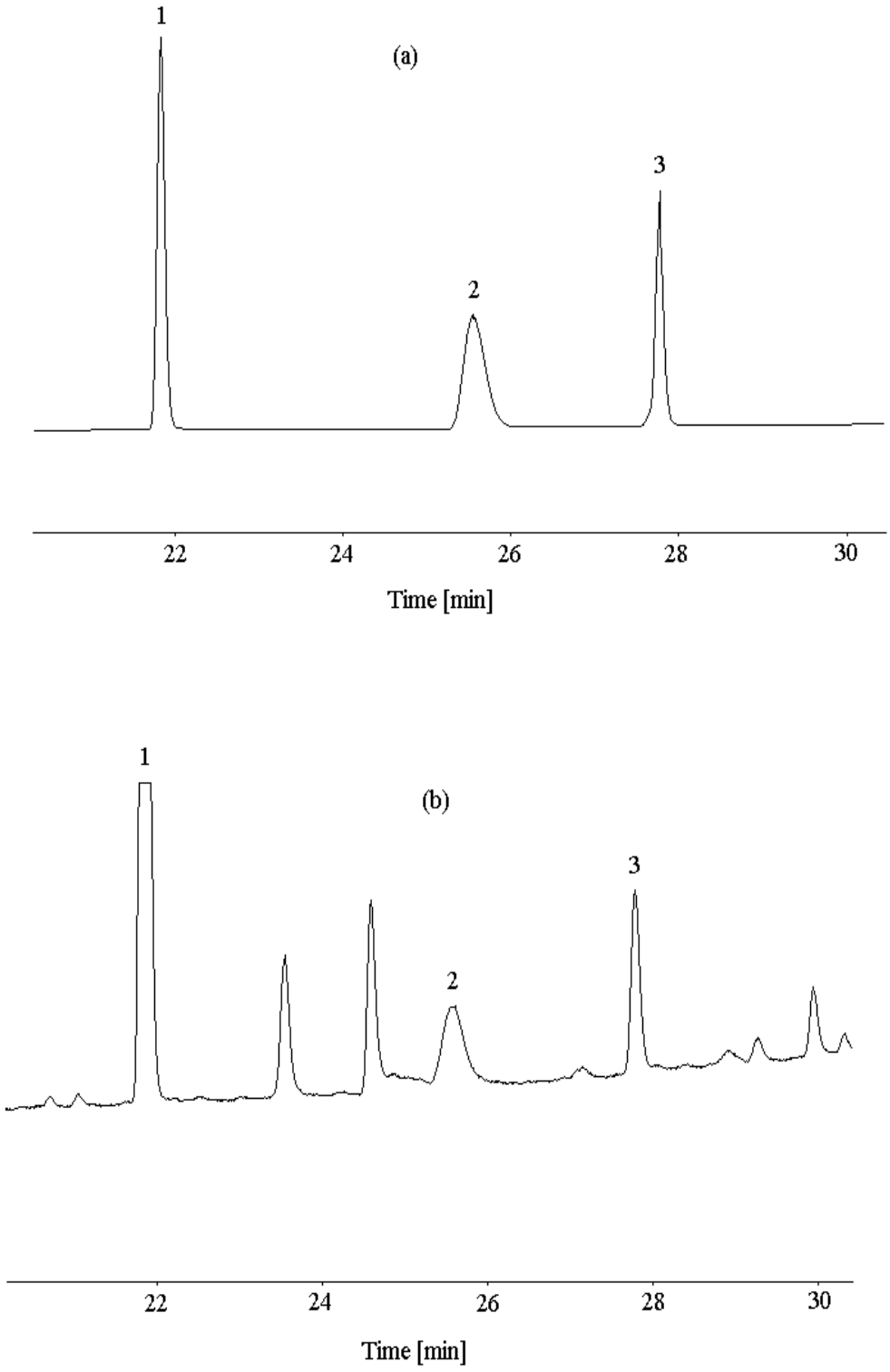


Figure 5.4 GC/NPD chromatograms of pirimicarb (1), PMC-D (2) and PMC-DF (3): (a) a standard solution at concentration of 5 ng/µL, (b) a soil sample extract from the field experiment at Nienwohlde 28 days after pirimicarb application.

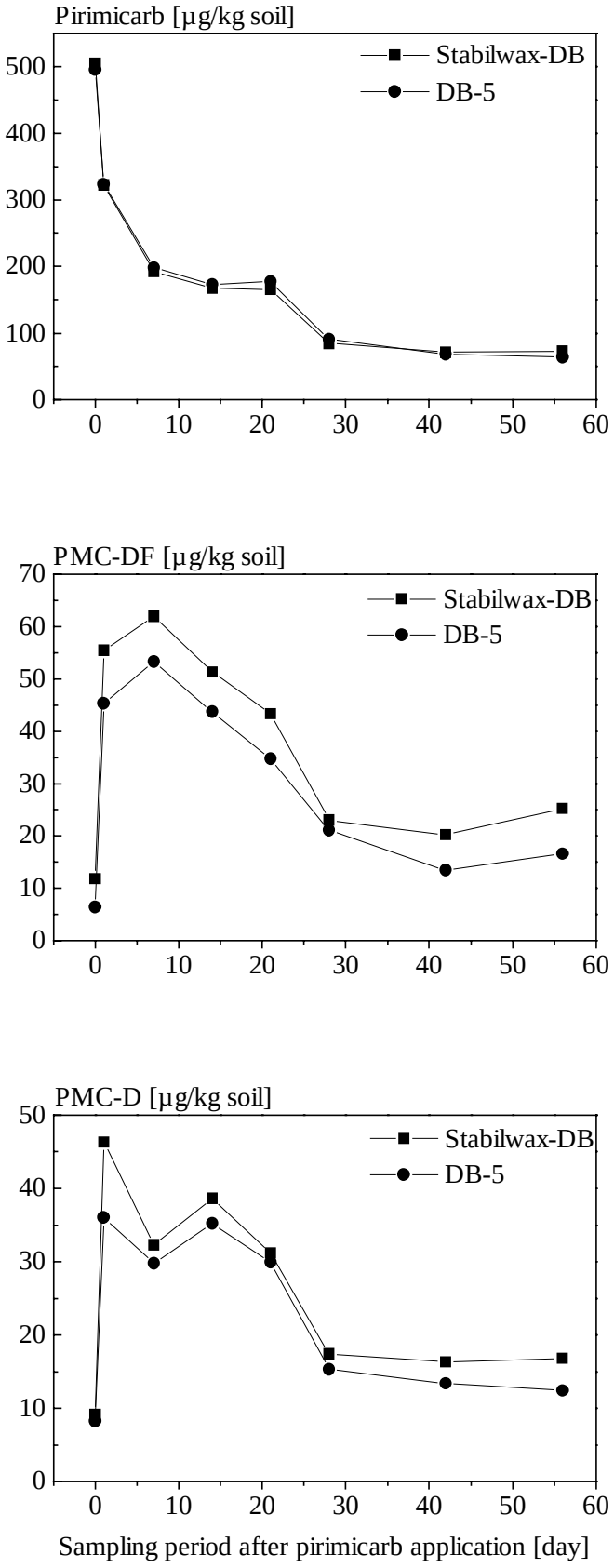


Figure 5.5 Comparison of quantitative results analyzed by GC/NPD with two capillary columns of different polarities: the Stabilwax-DB and the DB-5.

5.1.2 Recoveries and determination limits

The analytical method discussed above was evaluated with fortification experiments through determination of the recoveries (R %) and determination limits (LOD) for pirimicarb and the two metabolites PMC-DF and PMC-D. Experiments were performed with the clayey silt soil (NK-soil) and the silty sand soil (NW-soil), respectively. At first, blank soil samples were analyzed to make sure that the soil samples were not contaminated by the target compounds. Then, soil samples were spiked at different concentrations and analyzed in replicates.

According to the DFG concept (DFG 1991b), the following criteria have been defined for analytical method evaluation: average recoveries must be over 70 % by fortification experiments with 4 replicates, relative standard deviations of recoveries must be less than 20 %, and determination limits must be higher than the detection limits.

Recoveries of pirimicarb and its metabolites PMC-DF and PMC-D in the clayey silt soil acquired by HPLC/UV, GC/MS and GC/NPD measurements are listed in **Table 5.1**.

No blank values were detected for all three compounds. In a concentration range of 10 - 100 µg/kg, average recoveries varied between 88 and 101 % by HPLC/UV, 82 and 118 % by GC/MS, and 92 - 111 % by GC/NPD analysis. Standard deviations were all within 20 % in this concentration range. Although satisfactory recoveries were obtained for pirimicarb and PMC-DF at the spiking level of 5 µg/kg soil, this concentration level was quite close to the detection limit with regard to a signal/noise relationship of 3:1 in the HPLC/UV chromatograms. Thus, 10 µg/kg soil was set as the determination limit for all three target compounds by HPLC/UV analysis. By GC/MS analysis, except the recovery of PMC-D, which was too high at the concentration level of 5 µg/kg soil, satisfactory recoveries were obtained for pirimicarb and PMC-DF. Therefore, determination limits of 5 µg/kg for pirimicarb and PMC-DF and 10 µg/kg for PMC-D could be achieved by GC/MS analysis. For GC/NPD analysis a determination limit of 5 µg/kg could be reached for all three compounds.

Blank values as high as 21 ± 1 and 9 ± 1 µg/kg soil were determined from 6 blank samples by HPLC/UV analysis in the silty sand soil (NW-soil) for pirimicarb and PMC-DF,

Table 5.1 Recoveries (%) with standard deviation of pirimicarb, PMC-DF and PMC-D from spiked clayey silt soil samples, analyzed by HPLC/UV, GC/MS and GC/NPD, respectively.

Substance	Spiking level [µg/kg]	Recoveries [%]		
		HPLC/UV	GC/MS	GC/NPD (3)
Pirimicarb	5.0	121	110	95 ± 12
	10.0	92 ± 7 (1)	95 ± 12 (2)	94 ± 9
	20.0	-/-	-/-	94 ± 8
	50.0	88	102	92 ± 5
	100.0	90	102	-/-
	1000	101	-/-	-/-
PMC-DF	5.0	80	104	107 ± 11
	10.0	99 ± 12 (1)	104 ± 6 (2)	111 ± 2
	20.0	-/-	-/-	114 ± 13
	50.0	90	88	102 ± 6
	100.0	98	91	-/-
PMC-D	5.0	56	178	110 ± 17
	10.0	93 ± 12 (1)	118 ± 20 (2)	102 ± 2
	20.0	-/-	-/-	96 ± 10
	50.0	98	84	103 ± 8
	100.0	86	82	-/-

(1) analyzed in 8 replicates, others in duplicates

(2) analyzed in 4 replicates, others in duplicates

(3) analyzed in 4 replicates

-/- not analyzed

respectively. However, GC/MS and GC/NPD analyses revealed that these blank signals were not from pirimicarb and PMC-DF, but resulted from the soil matrix interference. Because of the interfering of the blank signals, problems were encountered during the evaluations of recoveries by HPLC/UV analysis for pirimicarb and PMC-DF at low fortification levels, whereas satisfactory recoveries of 118 and 94 % at the fortification levels of 5 and 10 µg/kg

soil, respectively, were obtained for PMC-D with matrix standards calibrations. Changing gradient programs or using HPLC/DAD analysis with subsequent manual integration did not bring any significant improvement. Acceptable recoveries were obtained for all three compounds over the fortification level of 50 µg/kg soil, i.e. 120 % for pirimicarb, 92 % for PMC-DF and 104 % for PMC-D.

In contrast, as shown in **Table 5.2**, satisfactory recoveries were obtained by GC/NPD analysis for pirimicarb and the two metabolites in the concentration range of 5.0 - 50.0 µg/kg with high repeatability (relative standard derivation ≤ 15 %).

Table 5.2 Recoveries (%) with standard deviation of pirimicarb, PMC-DF and PMC-D from spiked silty sand soil samples, analyzed by GC/MS and GC/NPD.

Substance	Spiking level [µg/kg]	Recoveries [%]	
		GC/MS	GC/NPD (1)
Pirimicarb	5.0	122, 100	98 ± 8
	10.0	104, 98	89 ± 11
	50.0	92, 90	87 ± 10
PMC-DF	5.0	80, 71	116 ± 10
	10.0	86, 84	100 ± 14
	50.0	92, 87	99 ± 15
PMC-D	5.0	120, 108	94 ± 7
	10.0	118, 116	87 ± 9
	50.0	82, 82	82 ± 11

(1) analyzed in 4 or 5 replicates

5.1.3 Soil sorption of pirimicarb and its metabolites

Determination of the soil/water distribution coefficients (K_d values) with batch equilibrium studies is the wide spread experimental method for the investigation of sorption properties of pesticides, in which the amount of pesticide adsorbed on soils are not directly measured but

calculated from the concentration decrease in soil solutions. Singh et al. (1990) and McCall et al. (1981) believed that other processes like degradation and volatilization, which contribute also to the concentration decline in soil solutions, are ignored in such indirect measurement. As a result, pesticides sorption on soils could be overestimated. They recommended the direct determination of pesticide concentrations in soil as well as in soil solution. However, because of the simplicity and quickness of the indirect method, it is often applied for a comparative primary evaluation of sorption behavior of one pesticide in different soil types or of different pesticides in the same soils.

Batch equilibrium studies are often carried out according to the OECD-guide line 106 (1981) or its modified variations. Because the soil/water ratio of 1:5 in the OECD-guide line 106 has been often criticized as not reflecting the natural soil/water relationship under field conditions and thus leads to K_d values notably higher than that with lower soil/water ratios (Stockmaier, 1996; Dibbern and Pestemer, 1992; Boesten, 1990), a soil/water ratio of 1:1.4 was used in this work according to a modified method reported by Stalder and Pestemer (1980) and Bunte (1991).

Distribution coefficients (K_d values) of pirimicarb and its metabolites PMC-DF and PMC-D in the clayey silt soil (NK-soil) and silty sand soil (NW-soil) are presented in **Table 5.3**.

In the clayey silt soil, similar leaching potential of the parent compound pirimicarb and its metabolites PMC-DF and PMC-D could be derived from their K_d values which were determined to be 2.0, 2.7 and 1.6, respectively. These low K_d values indicated that notably amounts of pirimicarb and the two metabolites are dissolved in the soil solution. As a result, they might be transported into deeper soil layers by infiltration water.

While the K_d values of PMC-DF in the silty sand soil were comparable to those in the clayey silt soil, remarkable differences existed in the K_d values of pirimicarb and PMC-D between the two soil types. Their K_d values were over 23 and 18 times greater in the silty sand soil than in the clayey silt soil, respectively. These results revealed that sorption of pirimicarb and PMC-D to the soil matrix should be much stronger in the silty sand soil.

Table 5.3 Distribution coefficients (K_d values) of pirimicarb and its metabolites PMC-DF and PMC-D in the clayey silt soil (NK-soil) and silty sand soil (NW-soil), analyzed by GC/NPD with DB-5.

Substance	Soil	Spiking level [µg/kg soil]	K_d value	Replicates
Pirimicarb	Clayey silt	50/50	2.0 ± 0.1	4
Pirimicarb	Silty sand	50/50	46 ± 4	4
PMC-DF	Clayey silt	10/10	2.7 / 2.7	2
PMC-DF	Silty sand	10/10	1.4 / 1.3	2
PMC-D	Clayey silt	10/10	1.6 / 1.5	2
PMC-D	Silty sand	10/10	27 / 30	2

Difference in the molecular structures between pirimicarb, PMC-DF, and PMC-D is only one functional group (see **Figure 3.1**). Thus, the different sorption behaviors of pirimicarb and PMC-D in the two soil types may be caused by their alkalinity and the differences in the soil properties. Rütters et al. (1999) studied the sorption behavior of the imidazole fungicide prochloraz in the same clayey silt soil and silty sand soil. They found that the pH values of the soils had considerable influence on the sorption behavior of the weakly basic prochloraz, given rise to a stronger sorption at lower pH values. Beside pH influence, soil organic carbon contents (C_{org}) had also positive correlation with the K_d values. Pirimicarb is a tertiary amine and PMC-D a secondary amine. The soil properties listed in **Table 2.1** show that the silty sand soil is acidic with a pH value of 5.3 and C_{org} of 1.52, whereas the clayey silt soil is neutral, having a pH value of 7.3 and C_{org} of 0.97. Therefore, the high K_d values of pirimicarb and PMC-D in the silty sand soil could be explained as a result of acid-base interaction in the binding of pirimicarb and PMC-D to the soil matrix, in combination with the positive effect of organic carbon content. The significance of such acid-base interaction has been also emphasized by Alzaga et al. (1995) who tried to determine pirimicarb residue in soil using supercritical fluid extraction (SFE). They have pointed out the relevance of pirimicarb binding onto acidic points of the soil matrix.

5.1.4 Behavior of pirimicarb and its metabolites in laboratory batch experiments

Laboratory batch experiments were carried out to investigate the degradation behavior of pirimicarb in the clayey silt soil and the silty sand soil. More important, the laboratory batch experiments should reveal whether the two metabolites PMC-DF and PMC-D would appear as degradation products of pirimicarb at detectable concentrations in the two soil types.

Clayey silt soil (NK-soil)

The results of the laboratory batch experiments of pirimicarb degradation in the clayey silt soil from the investigation site Neunkirchen (NK-soil) are depicted in **Figure 5.6**.

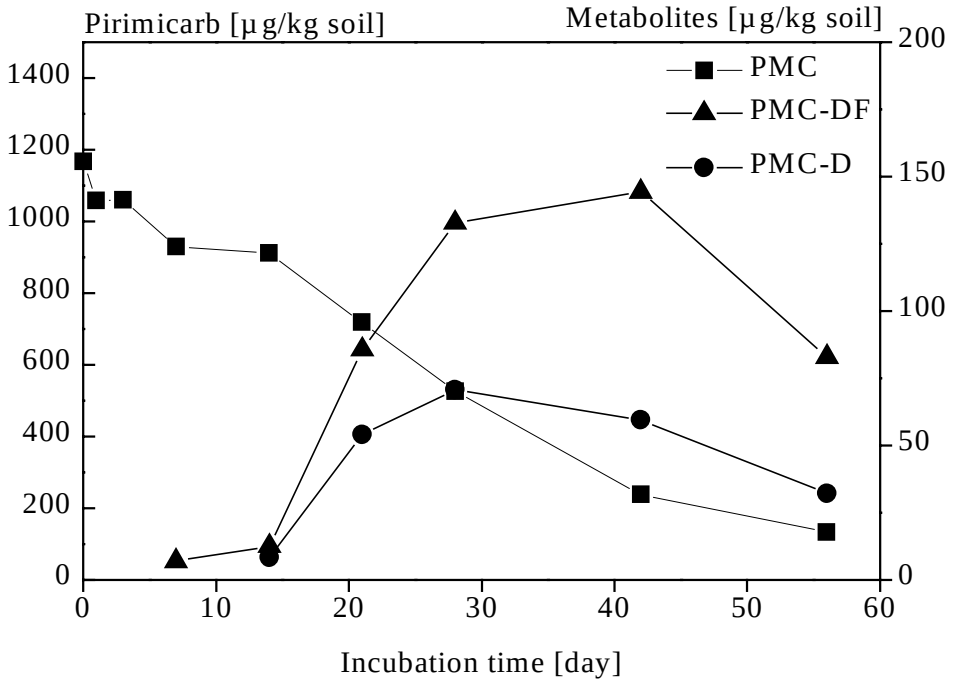


Figure 5.6 Concentrations of pirimicarb and the metabolites PMC-DF and PMC-D in a clayey silt soil in the laboratory batch experiments, analyzed in duplicates by HPLC/UV.

The initial concentration of pirimicarb spiked was 1150 µg/kg dry soil. Within the first 14 days of soil incubation, pirimicarb concentration decreased slowly. Then, the concentration decline became faster. At the end of the batch experiments, pirimicarb concentration reduced to 133 µg/kg soil, accounting for 11 % of pirimicarb initially applied. According to the calculation method described by Timme et al. (1986), a DT_{50} value of 16 days and a DT_{90}

value of 52 days were derived from the concentration decline of pirimicarb in this clayey silt soil. On the authority of the BBA-guidelines for pesticide registration, a pesticide having a DT_{50} value < 20 days and/or a DT_{90} value < 100 days can be regarded as not persistent and have no sustainable impact on the environment.

Table 5.4 Results of laboratory batch experiments of pirimicarb degradation in the clayey silt soil, determined by HPLC/UV and GC/MS.

Day after application	Pirimicarb [µg/kg]		PMC-DF [µg/kg]		PMC-D [µg/kg]	
	HPLC/UV	GC/MS	HPLC/UV	GC/MS	HPLC/UV	GC/MS
1	1058	1364	n.d.	n.d.	n.d.	10
3	1060	1236	n.d.	n.d.	n.d.	12
7	929	1024	n.q.	11	n.q.	18
14	910	1019	12	15	(8)	31
21	718	750	86	80	54	53
28	526	489	133	120	71	63
42	238	227	144	124	59	48
56	133	118	83	64	32	27

n.d. not detected

n.q. not quantified (< 10 µg/kg soil)

7 days after the application of pirimicarb signals at the retention time of the metabolites PMC-DF and PMC-D were detected by HPLC/UV analysis, but they could not be quantified. After a 14 days incubation period, the two metabolites could be first quantitatively determined at concentrations above the 10 µg/kg determination limit. This could be a result of the slow degradation of pirimicarb at the early stage of the experiments. Then, concentrations of the two metabolites, especially the primary formed degradation product PMC-DF, increased very fast. The PMC-DF concentration increased to a maximum of 133 µg/kg soil 42 days after soil application of pirimicarb, then rapidly decreased to 83 µg/kg soil in the last two weeks of the batch experiments. The concentration change of PMC-D was similar to that of PMC-DF. But PMC-D concentrations were generally lower than that of PMC-DF with a maximum value of 71 µg/kg soil reached 28 days after the soil incubation, then steadily decreased to 32 µg/kg soil at the end of the experiments. The detection of PMC-DF and PMC-D revealed that they

are degradation products of pirimicarb in soil. The subsequent concentration decreases of these two metabolites indicated their further degradation or transformation in soil.

The concentration determined by HPLC/UV were confirmed by GC/MS analysis. The results are shown in **Table 5.4**.

Silty sand soil (NW-soil)

The results of the laboratory batch experiments of pirimicarb degradation in the silty sand soil from the investigation site Nienwohlde (NW) were depicted in **Figure 5.7**.

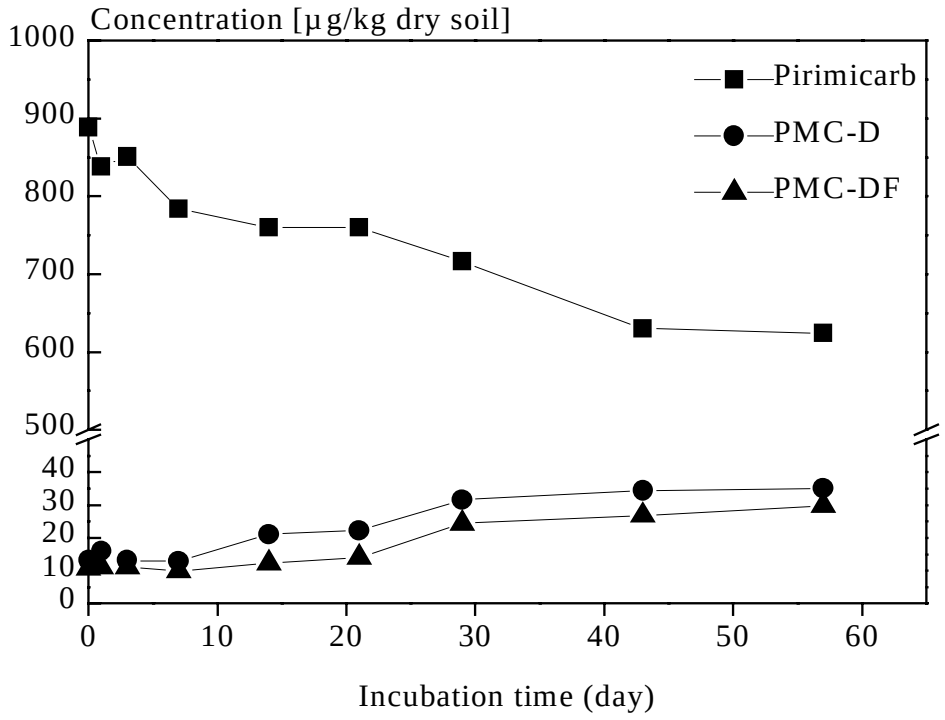


Figure 5.7 Concentrations of pirimicarb and the metabolites PMC-DF and PMC-D in a silty sand soil in the laboratory batch experiments, analyzed in duplicates by GC/MS.

The active substance pirimicarb was spiked to soil samples at an application rate of 1000 µg/kg dry soil. As shown in **Figure 5.7**, about 2 h after application the metabolites PMC-DF and PMC-D could be determined, respectively, at concentration levels of 13 and 11 µg/kg soil in the first duplicate soil samples without incubation. The immediate formation of the metabolites at this early stage is supported by the concentration decline of the active

substance pirimicarb from the initial concentration to an average value of 889 µg/kg soil. Because no metabolites were found in the pirimicarb standard solution used for the soil spiking, this could be only interpreted as a result of a immediate degradation of pirimicarb in the silty sand soil at this early stage. The degradation might be of microbial, chemical or photochemical natures.

However, further degradation of pirimicarb was unexpectedly slowed down. Pirimicarb showed a relatively high persistence in this silty sand soil from the investigation site Nienwohlde (NW). During the whole soil incubation period, pirimicarb concentration decreased successively. At the end of the batch experiment, 57 days after pirimicarb application, nearly 62 % of pirimicarb initially applied remained as extractable residues. A DT_{50} value of 213 days could be derived from the concentration decline of pirimicarb in the silty sand soil, indicating a high persistence of pirimicarb in this soil type. During the same time, concentration of PMC-DF and PMC-D gradually increased to about 30 and 25 µg/kg soil after 29 days incubation, respectively, and remained nearly constant with prolonged incubation.

The contrast between the fast degradation of pirimicarb at the experiment's beginning and the slow dissipation of pirimicarb during the whole soil incubation period indicated that the immediate degradation of pirimicarb within the first 2 h after its soil spiking might be mainly due to non-biological degradation reactions.

The two laboratory batch experiments were carried out with two different soil types under the same experimental conditions. The degradation of pirimicarb as well as the formation of the metabolites were significantly different. This clearly revealed that soil properties have strong influences on the behavior of pesticides and their degradation products in soil.

The different persistence of pirimicarb in the two soil types should have a direct relationship with the large difference in the K_d values of pirimicarb in the two soil types. It is well known that microbial degradation of pesticides in soil were generally regulated by two variables: the quantity of microbial biomass in the soil and the quantity of pesticide dissolved in the soil solution. The higher K_d value of pirimicarb in the silty sand soil indicated a stronger sorption of pirimicarb on this soil matrix and therefore, reduced concentration in the soil solution and decreased bioavailability. As a consequence, pirimicarb became more persistent in the silty

sand soil. Thus, in this study, the difference in pH values between the two soil types has the determining effect on the sorption and persistence behavior of pirimicarb.

No mass balance could be drawn between pirimicarb initially applied and the extractable residues of pirimicarb and its metabolites. Beside degradation reactions, formation of non-extractable residues plays an important role at the dissipation of extractable pirimicarb residues in soil. It has been reported that as many as 70 % of pirimicarb was determined as non-extractable residues 24 months after application (Agnihotri and Barooah, 1994).

5.1.5 Behavior of pirimicarb and its metabolites in field experiments

Information obtained from laboratory batch experiments are limited for the prediction of the fate of pesticides in the real environment. The behavior of a pesticide and its degradation products under natural field conditions during agricultural applications is of more interest and importance. However, factors affecting the behavior of pesticides can largely vary from soil to soil and from time to time under field conditions. It is, of course, also difficult to gain a reliable and substantial understanding.

As described in **Chapter 4.7**, field experiments were carried out at the two investigation sites Neuenkirchen (NK) and Nienwohlde (NW) independently in a plot within a sugar beet field within the same vegetation period. Pirimicarb was foliar-applied to the sugar beets as Pirimor® with doubled application rate to enable the determination of metabolites that possibly appeared at trace levels. In addition, Pirimor® was also directly sprayed to the bare soil surface of a field area in NK. This was aimed at getting a higher pirimicarb concentration in soil for the detecting of its metabolites.

During the field experiments in NK and NW, blank samples were taken from a neighbor sugar beet plot in each sampling time. They were prepared and analyzed together with the corresponding soil samples taken from the plot having pirimicarb application. No blank values of pirimicarb and its metabolites were found in any of these blank samples.

At the investigation site in Neuenkirchen (NK)

The field experimental results of pirimicarb directly applied to the soil are depicted in **Figure 5.8**.

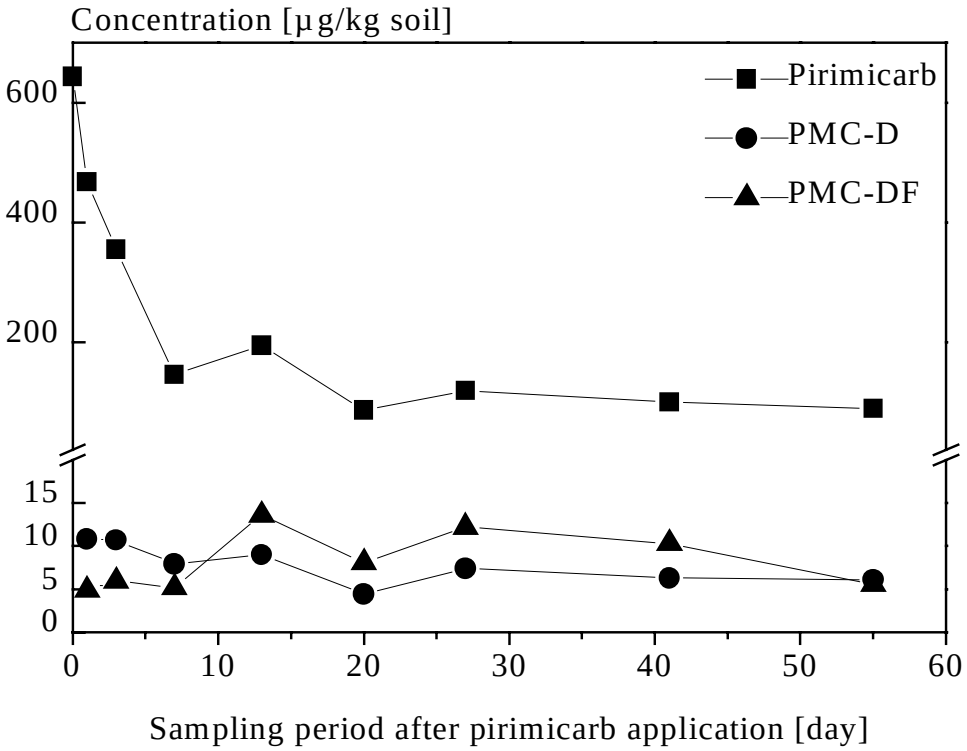


Figure 5.8 Concentrations of pirimicarb and the two metabolites PMC-DF and PMC-D in the superficial 0-5 cm soil layers in the field experiments at Neuenkirchen after direct soil application of pirimicarb, analyzed by GC/NPD with Stabilwax-DB.

In contrast to the dissipation pattern of pirimicarb in the laboratory batch experiment, pirimicarb disappeared quickly without any lag phase from 643 µg/kg soil to 147 µg/kg soil in the superficial 0-5 cm soil layers in the first week after pirimicarb application. This means almost 80 % pirimicarb disappeared at this early stage.

After the quick dissipation at the beginning the dissipation rate of pirimicarb sharply reduced, and 27 days after pirimicarb was applied, its concentration remained nearly constant around the level of 100 µg/kg soil. At the end of the field experiments, approximately 15 % of pirimicarb originally arrived at the soil surface remained as extractable residues. A DT_{50} value

of 1,1 days and a DT_{90} value of 18 days could be derived from the concentration decline of pirimicarb in the clayey silt soil of NK under field conditions.

At the early stage of the field experiments photo-degradations might significantly contribute to the fast disappearance of pirimicarb under the field conditions. As discussed in **Chapter 3.1**, pirimicarb can undergo a series of photo-degradation reactions. Unlike the laboratory batch experimental conditions, in which soil samples spiked with pirimicarb were incubated in the dark, the field conditions in the summer with intensive sun irradiation of the soil surfaces could possibly enable some photo-degradation reactions.

Beside photo-degradations, the rapid disappearance of pirimicarb could also result from accelerated microbial degradations due to the high summer soil temperature.

Volatilization of pirimicarb directly after the application could be another reason for the rapid dissipation of pirimicarb. Siebers et al. (1994) investigated the occurrence of pirimicarb and other pesticides in precipitation in northern Germany. But, they found that pirimicarb was rarely appeared in precipitation and its occurrence was mainly limited to the application period. Heath et al. (1992) also pointed out that volatilization of pirimicarb from soil surface was relatively moderate, only about 10 % of the originally applied disappeared 24 h after the application. Cabras et al. (1990, 1995) also suggested that volatilization did not represent the primary pathway of pirimicarb losses.

The obviously reduced dissipation rate of pirimicarb in the later experiment period could be interpreted as a result of reduced bioavailability of pirimicarb, when the adsorption of pirimicarb to soil matrix became stronger with the time and its concentration in soil solution reduced successively. Another possible explanation is reduced photo-degradations, when pirimicarb was washed from the soil surface into the soil layers by infiltration water after precipitation events, as indicated by its low K_d value of 2.0 and was also proven by pirimicarb residues determined in 5-10 cm soil layers in the field experiments.

The intense dissipation of pirimicarb in the first week was reflected partly by the formation of the two metabolites. In the soil sample taken from the superficial 0-5 cm soil layers 1 h after application, signals of the two metabolites, especially PMC-D, were already detectable, but they were still hardly to be quantified. One day after the application the concentration of

PMC-D reached 10 µg/kg soil, while the concentration of PMC-DF was only 5 µg/kg soil. Compared with the results in the laboratory batch experiment, this earlier formation of the two metabolites under field conditions should be an evidence of an immediate photo-degradation of pirimicarb shortly after application. In addition, the higher concentration levels of PMC-D at this early stage was also not in agreement with the results of the laboratory batch experiments, in which PMC-DF was the primary formed metabolite with concentrations always higher than those of PMC-D. Cabras et al. (1995) have pointed out that unlike the early presumption of FAO/WHO (1977) and Kopf (1992), PMC-DF did not change to PMC-D in their photo-degradation tests. Therefore, the higher concentration levels of PMC-D should be resulted directly from the photo-degradation of pirimicarb.

Later on, concentrations of the two metabolites were obviously lower under field conditions than that during the laboratory batch experiments. The concentration of PMC-DF increased slightly to about 15 µg/kg soil and remained further higher than that of PMC-D, while PMC-D remained at very low concentration levels near the determination limit. Cabras et al. (1995) have found that PMC-DF and PMC-D themselves undergoes photo-degradations, and PMC-D degraded faster than PMC-DF. The results of this field experiment seems to confirm this suggestion. Under field conditions, the degradation of the two metabolites continuously happened with the contribution of photo-induced degradations.

In order to study the vertical transport tendency of pirimicarb and the two metabolites, soil samples were taken from the 5-10 cm soil layers after the first heavy rainfall. The results are shown in **Table 5.5**.

The active substance pirimicarb was determined in the 5-10 cm soil layers with the highest concentration reaching a level of 64 µg/kg. This clearly resulted from the relatively high water solubility of pirimicarb (3000 mg/L water at 20 °C) in comparison with the thiocarbamate triallate (4 mg/L water at 25 °C), which has been reported to show negligible leaching into 5-10 cm soil layers under field conditions (Smith, 1970). The metabolites PMC-DF and PMC-D were only casually detected but could not be quantified in the 5-10 cm soil layers because of their low concentrations as degradation products of pirimicarb.

The concentration changes of pirimicarb residue in the soil after foliar application was similar to that after direct soil application and were presented in **Table 5.5**. As expected, the

concentrations of pirimicarb in soil were always lower after the foliar application than that after soil application, At the last two sampling times the clayey silt soil was so dry that sampling from the 5-10-cm soil layers become impossible with the bulb planter.

Table 5.5 Concentrations of pirimicarb residue in the field experiments at the investigation site Neuenkirchen (NK): Pirimor® application on June 27, 1995.

Sampling period [day]	Soil moisture [g/100g soil]	Pirimicarb [µg/kg dry soil]			
		foliar application		soil application	
		0-5 cm	5-10 cm	0-5 cm	5-10 cm
0	11.7	588	-/-	643	-/-
1	11.0	433	-/-	468	-/-
3	10.4	207	-/-	355	-/-
7	18.5	43	8.0	147	29
13	9.7	56	30	195	64
20	19.5	73	8.2	87	13
27	9.3	68	18	120	45
41	6.6	64	-/-	100	-/-
55	3.3	62	-/-	90	-/-

-/- not sampled

Although the metabolites PMC-DF and PMC-D could be detected in the superficial 0-5-cm soil layers after foliar application of pirimicarb, their amounts were too low to be quantified at most of the sampling periods. None of them could be found in the 5-10-cm soil layers.

The results of another field experiment in Neuenkirchen with pirimicarb foliar application are listed in **Table 5.6**. It confirmed the extensive dissipation of pirimicarb in the clayey silt soil. In addition, as high as 83 µg/kg soil of pirimicarb was found in the 5-10 cm soil layers, indicating the high tendency of pirimicarb transport through the superficial soil layers. The formation of its metabolites PMC-DF and PMC-D was also proven in this experiment.

Table 5.6 Concentrations of pirimicarb in the field experiments at the investigation site Neuenkirchen (NK): Pirimor® application on June 14, 1995.

Sampling period [day]	Pirimicarb [µg/kg soil]		PMC-DF [µg/kg soil]		PMC-D [µg/kg soil]	
	0-5 cm	5-10 cm	0-5 cm	5-10 cm	0-5 cm	5-10 cm
13	231	83	10.4	n.q.	12.5	6.4
26	155	55	10.4	n.q.	13.5	5.2
54	136	-/-	15.8	-/-	19.6	-/-

n.q. not quantified

-/- not sampled

Similar to the laboratory batch experiments, the large spans between concentrations of pirimicarb disappeared and the metabolites formed indicated that other processes such as volatilization, microbial or photo-degradation to other metabolites and the formation of non-extractable residues should contribute to pirimicarb dissipation in the field as well.

At the investigation site Nienwohlde (NW)

The field experimental results after foliar application of pirimicarb at the investigation site Nienwohlde (NW) are depicted in **Figure 5.9**.

Contrary to the results of the laboratory batch experiments with the silty sand soil of NW, but similar to the situations in the field experiments at Neuenkirchen, pirimicarb disappeared rapidly in the superficial 0-5 cm soil layers from the initial concentration of 504 µg/kg soil to 192 µg/kg soil in the first week after application. Then, its dissipation rate paced down. At the end of the experiments, 72 µg/kg soil of pirimicarb remained as extractable residues in the silty sand soil. A DT_{50} value of 3 days and a DT_{90} value of 26 days could be derived from the concentration decline of pirimicarb in this silty sand soil.

PMC-DF and PMC-D were already determined at the concentrations of 12 and 9 µg/kg soil, respectively, in the superficial 0-5 cm soil layers from the first sampling in NW. PMC-DF concentration increased to a maximum of 62 µg/kg 7 days after application, then decreased

gradually to 23 µg/kg soil in the following weeks and remained nearly constant until the end of the experiments. Concentration changes of PMC-D were similar to that of PMC-DF but were generally lower, with its maximum reached already 1 day after the pirimicarb application. The concentration curves of the metabolites indicated their continuous formation and further degradation in the silty sand soil.

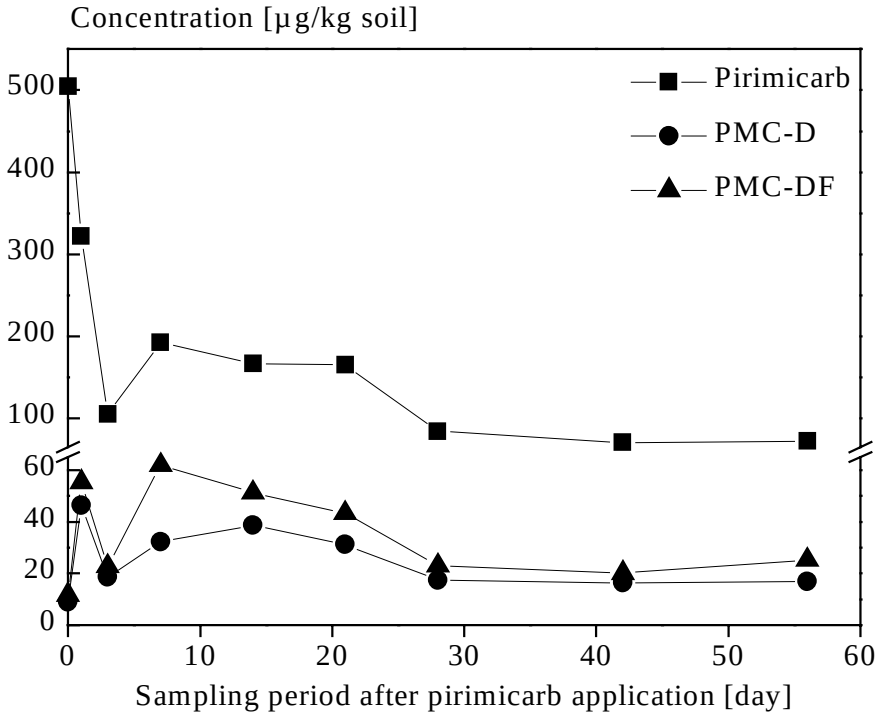


Figure 5.9 Concentrations of pirimicarb and the two metabolites PMC-DF and PMC-D in the superficial 0-5 cm soil layers in the field experiment at Nienwohlde after foliar application of pirimicarb, analyzed by GC/NPD with Stabilwax-DB.

Despite a higher persistence of pirimicarb in the silty sand soil, as shown by its K_d value (see **Table 5.3**), concentrations of the two metabolites formed in this soil were obviously higher than those in the clayey silt soil. This contradiction might indicate that under the field conditions microbial degradation played only a subordinate role, whereas photo-degradation contributed more to the formation of the metabolites, and the soil surface of the silty sand favored the photo-degradation processes. Additionally, the higher persistence of PMC-D in the silty sand soil, as indicated by its K_d values, might reduced the rates of its further transformation as well as its precursor PMC-DF.

As shown in **Table 5.7**, pirimicarb residues were determined in the 5-10 cm soil layers at all sampling times after the first precipitation event. Its concentration reached the highest value of 55 µg/kg soil 7 days after pirimicarb application, as a consequence of the vertical transport by infiltration water after heavy rainfalls. Then, the concentration decreased to about 7 µg/kg soil at the end of the experiment. At the later experimental stage, vertical transport of pirimicarb was not observed, perhaps as the result of stronger adsorption of pirimicarb to the soil matrix, as well as reduced concentration of pirimicarb in the superficial 0-5 cm soil layers.

Table 5.7 Concentrations of pirimicarb and its metabolites PMC-DF and PMC-D in the 5-10 cm soil layers in the field experiments at the investigation site in Nienwohlde (NW).

Sampling period [day]	Soil moisture [g/100 g]	Pirimicarb [µg/kg soil]	PMC-DF [µg/kg soil]	PMC-D [µg/kg soil]
0	7.9	-/-	-/-	-/-
1	7.7	-/-	-/-	-/-
3	10.4	14	n.q.	n.q.
7	6.1	55	16	n.q.
14	8.4	17	5.7	n.q.
21	5.4	31	10	7.8
28	7.6	23	5.6	6.4
42	10.1	7.0	5.5	n.q.
56	9.6	7.5	6.6	n.q.

n.q. not to be quantified

-/- not sampled

According to its K_d values determined, pirimicarb would be expected to be much less mobile in the silty sand soil than in the clayey silt soil. However, pirimicarb was found at similar concentrations in the 5-10 cm soil layers at both investigation sites. Since no deeper soil layers were sampled, an precise estimation of the mobility of pirimicarb in these two different soil types is not possible.

The metabolites PMC-DF and PMC-D were formed only at low concentrations. So their appearance in the 5-10 cm soil layers were around the determination limits in the silty sand soil and could be hardly quantified in the clayey silt soil.

5.2 Triallate and its metabolite TCPSA

5.2.1 Analytical method development

Preliminary tests carried out with water samples spiked with triallate and TCPSA have shown that the DFG S19 multi-residues method cannot be adapted directly for the extraction of TCPSA from water sample into organic solvent. No TCPSA was detected in the organic phase even after the aqueous phase was acidified to pH < 1 to prevent its dissociation in water before the liquid-liquid partition with dichloromethane. This clearly indicated that TCPSA is too polar to be extracted into a water-nonmiscible organic solvent. According to the BBA method (BBA, 1989), a modification of the method by using tetramethylammonium hydrogensulfate (TBAHS) as phase transfer catalyst was necessary for transferring TCPSA into a middle polar organic solvent such as dichloromethane. TBAHS is an often used phase transfer catalyst for ion pair extraction of various water-soluble organic substances from aqueous solutions into organic phases, as reported for N-ethylperfluorooctanesulfonamide and its metabolite perfluorooctanesulfonamide (Arrendale et al., 1989).

Before its methylation with trimethyl orthoformate to facilitate GC/ECD analysis, TCPSA was converted to its free acid form by dissolving the tetramethylammonium salt in methanol and passing through a cation exchange resin AG 50-WX-8. The eluates from the cation exchange resin were, however, yellow colored, indicating a bleeding of resin material. The color turned even darker after the methylation of the eluates. Many unidentified peaks appeared in the chromatogram by GC/ECD analysis. A column clean-up step using packed florisil column was necessary.

Due to the complexity of the solvent extraction procedure and problems encountered even with simply fortified water samples, the recovery of TCPSA from spiked water samples was found normally less than 70 %. With spiked soil samples additional problems appeared, such as the formation of stable emulsions at the liquid-liquid partition step, which were very

troublesome for further sample preparation. Additionally, the conventional solvent extraction procedure is labor-intensive, time-consuming and requires a relatively large volume of solvents.

Solid phase extraction (SPE) procedures possess some obvious advantages over the conventional solvent extraction. It consumes less solvent and needs generally shorter sample preparation time. Solid phase extraction combines extraction and enrichment of pesticide residues in one step. This means not only a simplification of sample preparation procedure, but also an enhanced determination sensitivity for pesticide residue analysis, since the enrichment factors can be significantly higher than that of conventional solvent extraction. Furthermore, some coextractants can be eliminated through selective elution of the SPE cartridges before the elution of target compounds. Thus, additional clean-up steps are usually not needed. Therefore, a sample preparation method based on solid phase extraction was developed in this work as an improved alternative for the sensitive determination of triallate and, especially, TCPSA in water and soil samples.

A flow chart of the analytical method developed in this work was illustrated in **Figure 5.10** and discussed in details as follows.

5.2.1.1 solid phase extraction

According to Aga et al. (1994) the herbicide alachlor and its sulfonic acid metabolite can be simultaneously extracted from water samples by a single C_{18} cartridge. However, the first approach using C_{18}-cartridges to enrich triallate and TCPSA from spiked water samples indicated that triallate was quantitatively retained, whereas TCPSA was apparently too polar to be retained.

Strong anion exchange (SAX) columns like the quaternary amine (NR_4^+) extraction column appear to be a proper choice for isolation of strong acids from aqueous matrices. NR_4^+ solid phase extraction cartridges have been applied for the isolation of aromatic sulfonic acids and linear alkylbenzenesulfonates from industrial waste water, sediments, sludge, and soils (Bastian et al., 1994; Nitschke and Huber, 1992; Berna et al., 1989; Castles et al., 1989; Matthijs and De Hanau, 1987; De Henau et al., 1986). Recently, SAX extraction disks were

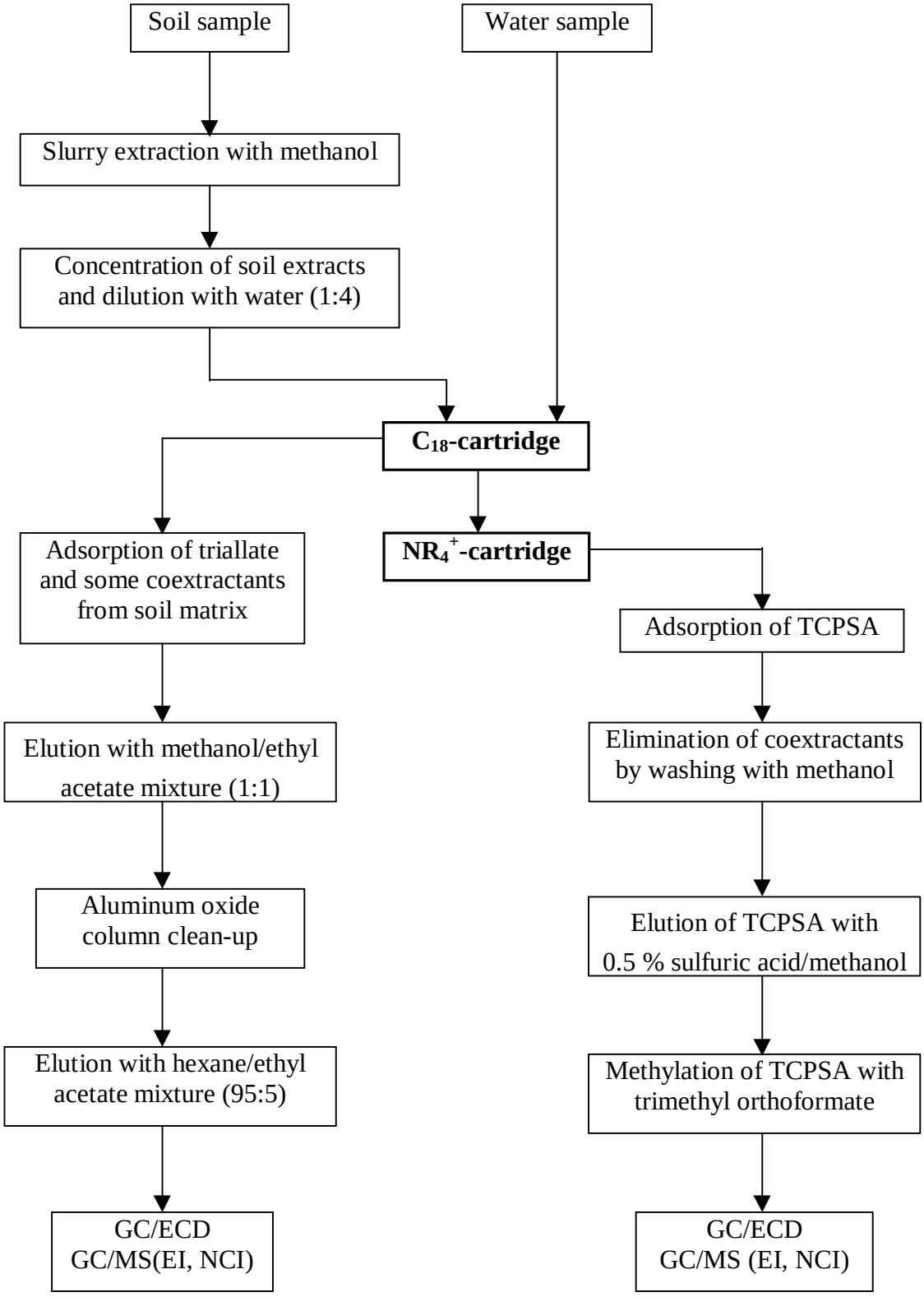


Figure 5.10 Flow chart for the analytical determination of triallate and TCPSA in water and soil samples (Wang et al., 1998a and 1998b)

also used to extract the herbicide Dacthal and its mono- and dicarboxylic acids as an alternative to conventional liquid-liquid extraction (Monohan et al., 1995). Therefore, a second approach was the attempt to extract TCPSA with NR_4^+-cartridges. The results showed that TCPSA could be quantitatively retained by NR_4^+ cartridges. As a consequence, a combination of the C_{18}- and NR_4^+-cartridges is needed to separately extract triallate and TCPSA.

The solid phase extraction procedure containing the following steps was developed for the extraction of triallate and its polar metabolite TCPSA from water samples and soil extracts: (1) pre-condition of the C_{18} and NR_4^+ extraction cartridges; (2) enrichment of water samples or soil extracts on the extraction cartridges; (3) selective rinse of the extraction cartridges to eliminate coextractants and (4) selective elution of the target compounds. The optimal experimental parameters were described in **Chapter 4.4.2**.

During the selection of a proper eluent for the elution of triallate from the C_{18}-cartridges, methanol alone was found to be too polar to achieve a quantitative elution of triallate. A modification of the eluent polarity by adding a moderately polar solvent like ethyl acetate was needed. Therefore, methanol/ethyl acetate 1:1 (v:v) was used to release triallate from the C_{18}-cartridges (triallate fraction). In the case of soil samples some coextracatives were unavoidably co-eluted in the triallate fraction, as well. They should be, subsequently, eliminated on an aluminum oxide column.

Analytes retained on an ion exchange column could be eluted using an eluent containing a high concentration of competing counter-ions or by adjusting the pH of the eluent to change the charge on the analytes or extraction columns. Methanol/HCl 20:5 (v/v) has been reported to be used for the elution of linear alkylbenzenesulfonates from anion exchange columns (Matthijs and De Hanau, 1987; De Henau et al., 1986). Therefore, during the selection of a proper eluent for the elution of TCPSA from the NR_4^+-cartridges, methanol/HCl 20:5 (v:v) was initially tested. However, it was found that HCl was not only corrosive against the metal stopcocks equipped in the Baker solid phase extraction system (spe* 12G), but also caused problems at the next derivatization step. Therefore, 0.5 % sulfuric acid in methanol was then chosen in this work after a series of tests. The low sulfuric acid concentration was proven to be strong enough for changing the charges on the analytes or extraction columns to release TCPSA from the NR_4^+-cartridges (TCPSA fraction). At the method development stage, to

make sure that TCPSA was quantitatively eluted by the first 5 mL of the 0.5 % sulfuric acid/methanol mixture, additional 5 mL of this eluent mixture and 5 mL of 2 % sulfuric acid/methanol were allowed to pass through the NR_4^+-cartridges. No TCPSA was, however, detected in these additional eluting fractions.

5.2.1.2 Soil sample extraction

Water samples can be directly treated with solid phase extraction. Soil samples must be first extracted with a suitable organic solvent or a solvent mixture to get soil extracts containing the target compounds. The soil extracts generally needed to be further treated to enable the solid phase extraction. Compared between methanol and acetone/water 2:1 (v:v) mixture for the extractions of soil samples, methanol was found to be the proper solvent for the extraction of triallate and TCPSA with high recoveries but relatively less coextractants from soil matrices. This was indicated by the color of soil extracts obtained which was obviously lighter from methanol extraction than that from extractions with acetone/water 2:1 (v:v) mixture. 100 mL methanol was proven to be sufficient for the quantitative extraction of triallate and TCPSA from 50 g soil sample within a large concentration range. After extraction and filtration, soil extracts were concentrated through evaporation of excess methanol and then diluted with demineralized water to give a methanol/water ratio of 1:4 (v:v) to facilitate the subsequent solid phase extraction. The remaining methanol was necessary for holding triallate dissolved in the soil extracts. This methanol/water ratio was tested out to be the right one to have clear soil extracts but still prevent a possible breakthrough of triallate from the C_{18}-cartridges.

5.2.1.3 Optimization of the derivatization of TCPSA

Without derivatization TCPSA was reported to be determined by reversed phase high performance liquid chromatography with UV-detection (Nadeau et al. 1993). Aga et al. (1994) and Mattern et al. (1995) have also used HPLC/UV for the determination of sulfonic acid metabolites of alachlor and phenol sulfonic acid of fenamiphos, respectively. However, HPLC/UV analysis of TCPSA conducted in this work indicated that TCPSA rapidly eluted from a C_{18} reversed-phase separation column. A reasonable retention time of TCPSA on the RP-C_{18} column was only achievable by using a mobile phase with very poor solvent eluting strength such as 5 % CH_3CN in 0.005 M KH_2PO_4 buffer (pH 7.0). However, under these

conditions, TCPSA peak could not be separated from the peaks of many polar coextractants from the soil matrix. More importantly, the slope of calibration curves showed that the sensitivity of HPLC/UV detection for TCPSA was at least one order of magnitude lower than that of GC/ECD detection for TCPSA methyl ester. This was essential, since TCPSA was expected to appear at trace levels as a degradation product of triallate. Therefore, further optimization of HPLC analysis such as the use of ion-pair liquid chromatography (Mattern et al., 1995) was not pursued. HPLC/UV was used in this work for the evaluation of methylation degrees of TCPSA but not for its determination in water and soil samples.

TCPSA was too polar for gas chromatographic separation. To take the advantage of the 3 Cl atoms existing in the TCPSA molecule, TCPSA was transformed to its volatile methyl ester to facilitate the sensitive GC/ECD determination.

Generally, the requirements of developing a derivatization method are to find a reaction with following characteristics: (1) it is quantitative or at least reproducible; (2) it converts the analyte to a single stable product; (3) it utilizes reagents that is suitable for a wide concentration range of the analyte; (4) the excess reagents can be relative easily separated from the final product and (5) coextractants from sample matrix, whether they are derived as well or not, must not interfere the determination of the analyte, or they can be removed from the sample by simple clean-up measures (Thier & Frehse, 1986).

Different kind of derivatives of sulfonic acids have been reported to be volatile enough for GC analysis, such as silylated derivatives (Ng and Hupé, 1990; Stokke and Helland, 1978; Eagles and Knowles, 1971; Caldwell and Tappel, 1968), sulfonic chlorides (Amer et al., 1982; Heywood, et al., 1970; Kirkland, 1960); sulfonamides (Tang and Wang, 1991) and methyl esters (Padmapriya et al., 1985; Kirkland 1960). Methylation of TCPSA with diazomethane was reported to be unsuccessful, neither was silylation (Ebing and Schuphan, 1978). Additionally, silylated derivatives usually suffer from the problem of instability due to hydrolysis. The use of diazomethane is associated with the problem of dealing with a high toxic, cancerogenic substance. The methods reported to get sulfonic chlorides or sulfonamides with two steps of reactions seemed not suitable for the derivatization of trace amount of sulfonic acids. Therefore, comparing the different derivatization methods with respect to the general requirements mentioned above, the simple method first reported by Padmapriya et al. (1985) appeared to be most suitable for methylation of trace amount of sulfonic acids. With

this method, sulfonic acids could be smoothly converted to their corresponding methyl or ethyl esters by reacting with trimethyl or triethyl orthoformate.

According to the method of Padmapriya et al. (1985), TCPSA was transferred to its volatile methyl or ethyl ester with trimethyl- or triethyl orthoformate (Nadeau et al., 1993; BBA, 1989). Methylation was chosen in this work, because ethylation of TCPSA was found to need reaction temperatures much higher than 100 °C and was not easy to handle with. To achieve an optimum derivatization of TCPSA, factors affecting the methylation reaction such as reaction temperature, reaction time and reaction medium were investigated in this work with respect to the sample matrices. It was desirable that methylation of TCPSA can be realized under mild conditions at possible low reaction temperature and within a short reaction time. Padmapriya et al. (1985) reported that p-methyl benzoic acid could be quantitatively methylated by standing in excess trimethyl orthoformate for 14 h at room temperature. However, no notable methylation of TCPSA was achieved under these conditions. In this work, a methylation of TCPSA at 95 ± 2°C for 2.5 h in 1 mL of the derivatization reagent trimethyl orthoformate without the addition of any solvents was found to be the best and simple way to obtain high yields of TCPSA methyl ester with possible less by-products.

The reaction mechanism has been suggested to be pH-controlled as depicted in **Figure 5.11**.

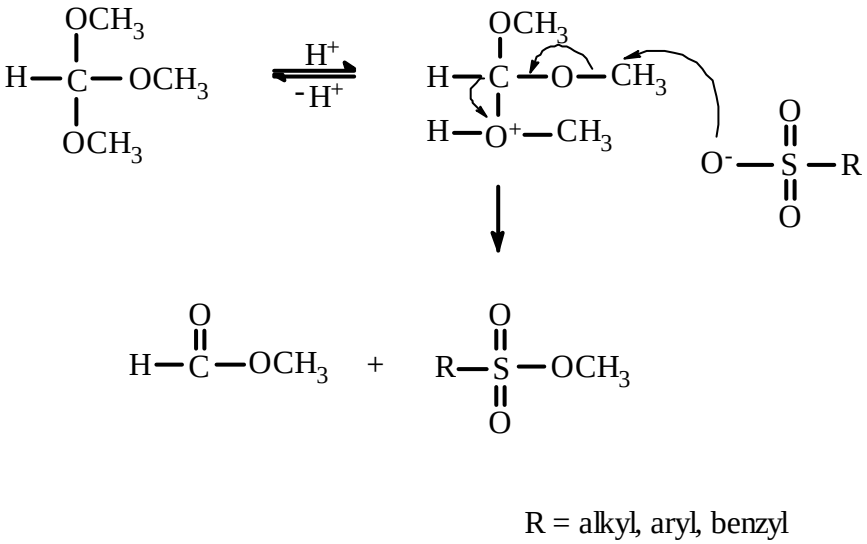


Figure 5.11 Suggested pathway of methylation for sulfonic acids with trimethyl orthoformate (according to Padmapriya et al., 1985)

As described in **Chapter 4.4.2**, TCPSA was eluted from NR_4^+-cartridges with 0.5 % sulfuric acid/methanol (TCPSA fraction). Methanol was totally evaporated from the eluates before derivatization, while sulfuric acid remained in the residues. To find out the effect of the remaining sulfuric acid on the derivatization, 5 mL methanol and 5 mL 0.5 % sulfuric acid/methanol were added to TCPSA standards. After methanol was evaporated, the acidified standards were methylated with trimethyl orthoformate. The results indicated that the remained sulfuric acid enhanced the methylation degree of TCPSA and the yield of TCPSA methyl ester increased averagely by approximate 50 %. HPLC/UV analysis showed that about 40 % of the originally added TCPSA remained unreacted, when TCPSA standards were methylated without sulfuric acid. This confirmed the suggested pH-controlled pathway for the methylation of TCPSA with trimethyl orthoformate.

After the methylation and the removal of excess derivatization reagent, a micro liquid-liquid partition with 1 mL hexane and 1 mL demineralized water was employed for a quantitative, or at least reproducible transfer of TCPSA methyl ester from the derivatization residues into the organic phase. This partition step was also necessary for the elimination of sulfuric acid to avoid its destroying influence on the GC performance. The yields of TCPSA methyl ester obtained with such micro liquid-liquid partition were found normally about 50 % higher than that obtained by a direct extraction of derivatization residues with hexane. The addition of 1 mL water had obviously forced more TCPSA methyl ester to be transferred into the hexane phase, while water-soluble substances in the derivatization residues were remained in the aqueous phase. The methylated TCPSA standards remained stable at least over two months if they were stored at -20 °C.

A series of TCPSA standards in the concentration range of 0.01 – 5.0 ng/µL were routinely methylated for recording calibration curves for the quantitative analysis of TCPSA methyl ester by GC/ECD. The repeatability of the methylation, described as the average deviation derived from repeated methylation of TCPSA standards at different concentrations, was generally within 10 %. However, higher deviation appeared from time to time. Therefore, new TCPSA methyl ester standards were prepared every time with the soil or water samples to be analyzed. Good linearity between peak areas of TCPSA methyl ester and TCPSA concentrations was guaranteed with this methylation method. Correlation coefficients were ≥ 0.999 in the concentration range of 0.01 - 0.5 ng/µL and ≥ 0.996 in the concentration range of 0.1 - 5.0 ng/µL.

Compared with the BBA method for TCPSA determination (BBA, 1989), the new method developed in this work based on solid phase extraction and subsequent methylation of TCPSA is significantly effective with considerably less solvent consumption and remarkably shorter sample preparation time.

5.2.1.4 Identification of TCPSA methyl ester

To identify TCPSA methyl ester obtained by the methylation with trimethyl orthoformate, GC/MS analysis with EI and NCI detection in full scan mode was performed. An EI mass spectrum of a methylated 100 ng/µL TCPSA standard and an NCI mass spectrum of a methylated 5 ng/µL TCPSA standard are shown in **Figure 5.12** and **Figure 5.13**.

The molecular ion $[M]^+$ m/z 238 of TCPSA methyl ester was detected in the EI mass spectrum with a relative abundance of about 20 %. The molecular ion cluster by m/z 238/240/242 and the fragmentation ion cluster by m/z 143/145/147 characterized the same isotope ratio that is typical for ions containing three Cl atoms. The occurrence of the fragmentation ion cluster by m/z 143/145/147 indicated the existence of 2,3,3-trichloroallyl ion resulted from the cleavage of C–S bond in the molecule of TCPSA methyl ester. Positive identification of TCPSA methyl ester was realized, because the existence of the ion clusters m/z 238/240/242 and m/z 143/145/147 in a same mass spectrum was found only for the target compound by the full scanning.

The NCI mass spectrum of TCPSA methyl ester showed an intensive fragmentation. No molecular ion $[M]^-$ was detected due to dissociative electron capture. The basic ion was m/z 95 which represents the methyl sulfonic group resulted also from the cleavage of C–S bond in the molecule of TCPSA methyl ester after the loss of the 2,3,3-trichloroallyl group. This is an additional evidence for the positive identification of TCPSA methyl ester.

When calibrating with the ion abundance of m/z 143 vs. the concentrations of TCPSA, a high linear relationship ($r = 0.998$) was obtained in the concentration range of 0.25 to 5.0 ng/µL. But the sensitivity of GC/MS analysis was nearly one order of magnitude lower than that of GC/ECD analysis.

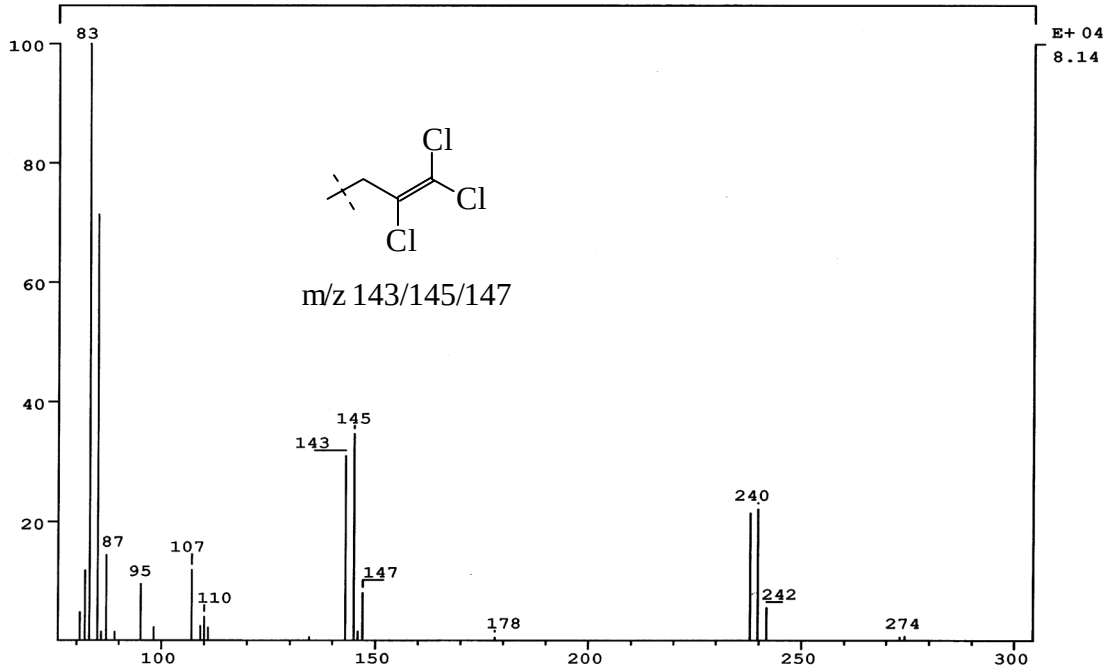


Figure 5.12 EI mass spectrum of TCPSA methyl ester (100 ng/µL).

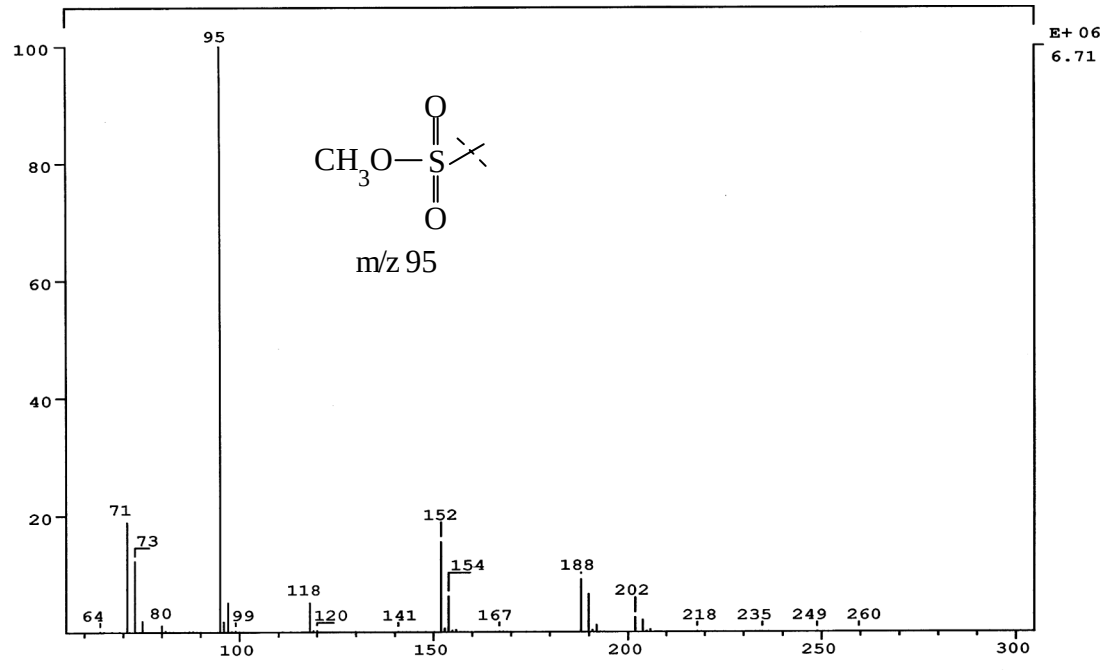


Figure 5.13 NCI mass spectrum of TCPSA methyl ester (5 ng/µL).

5.2.1.5 Recoveries and determination limits

Water and soil samples spiked with triallate and TCPSA to give different concentration levels were analyzed. The efficiency and repeatability of the analytical method developed were assessed in terms of recoveries and relative standard deviations. The lowest spiking concentration with satisfactory average recovery was set as the determination limit.

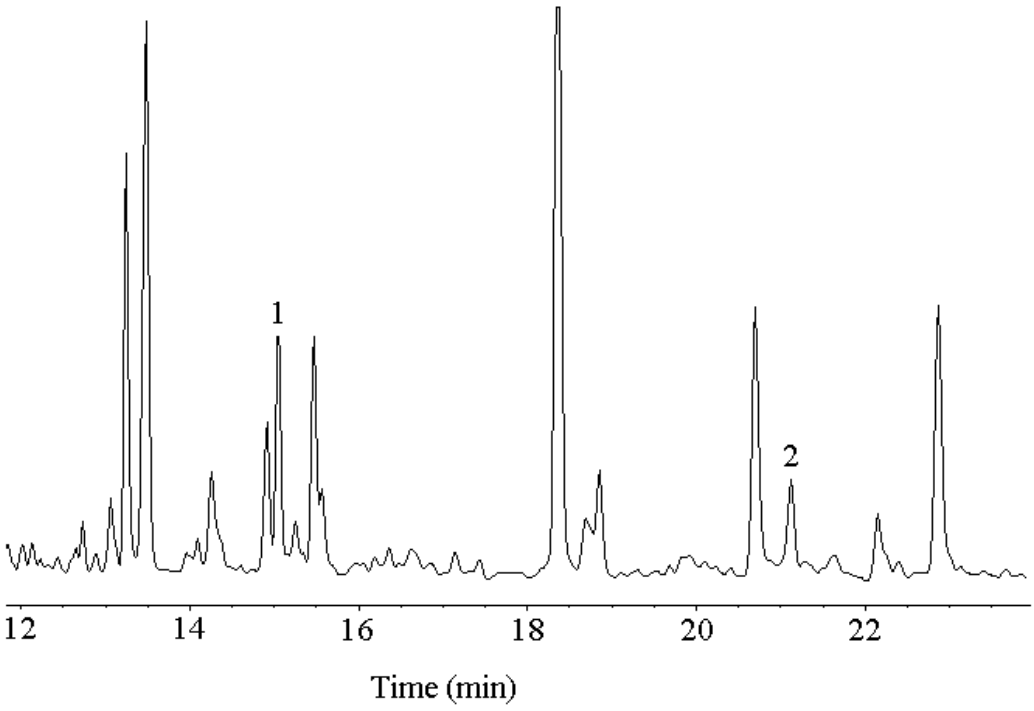


Figure 5.14 Simultaneous GC/ECD analysis of TCPSA (1) and triallate (2) in a soil extract from a fortification experiment. Concentration of both analytes was 10 µg/kg soil and GC temperature program was 60 °C → 15 °C/min → 170 °C → 4 °C/min → 230 °C → 20 °C/min → 280 °C (15 min)

As shown in **Figure 5.14**, triallate and TCPSA could be simultaneously analyzed by GC/ECD. However, to do this, aliquots of the purified triallate fraction should be combined with the methylated TCPSA-fraction. This means not only additional work but also increased interference of sample matrices for the determination of triallate and TCPSA. On the other side, the automation of gas chromatographic analysis enables a great number of samples to be analyzed overnight. Therefore, triallate and TCPSA were analyzed separately in this work.

Water samples. Water samples spiked with triallate and TCPSA were prepared according to the analytical method developed. A blank sample was always treated together with spiked samples to control the sample preparation procedures. By GC/ECD analysis no triallate was detected in blank samples. Blank signals of TCPSA were found from five blank samples but all at very low levels, namely, as an average, 4 times lower than the peak areas of TCPSA obtained from samples with the lowest spiking concentration of 0.05 µg/L. For spiked water samples high recoveries with good repeatability were obtained, as shown in **Table 5.8**. Average recoveries for triallate and TCPSA in the concentration range of 0.05 - 5.0 µg/L were 90 ± 12 % and 98 ± 14 %, respectively. A detection limit for triallate and TCPSA was then set at 0.05 µg/L for a water sample size of 400 mL.

Table 5.8. Recoveries (%) with standard deviations of triallate and its metabolite TCPSA from 400 mL spiked demineralized water samples.

Spiking level [µg/L]	Recoveries of triallate [%]	Recoveries of TCPSA [%]	Replicates [n]
0.05	84 ± 14	100 ± 22	9
0.10	85 ± 6	99 ± 9	6
0.50	93 ± 5	101 ± 14	8
5.0	99 ± 13	93 ± 4	3

Soil samples. Blank values of the triallate and TCPSA were determined in the silty sand soil from Nienwohlde (NW) and in the loamy sand soil from Sickte (SIC). For each soil type, four replicates were analyzed. By GC/ECD analysis with a DB 5 capillary column, no triallate blank signal was detected in the silty sand soil, whereas 2.6 µg/kg soil of triallate were detected as blank signal in the loamy sand soil. Blank signals of TCPSA were determined as 1.9 ± 1.0 and 2.8 ± 1.3 µg/kg soil in the silty sand soil and the loamy sand soil, respectively. However, the blank signals were proven to be resulted from interference of coextractants from the soil matrices, since no blank signals of triallate or TCPSA were detected by GC/ECD analysis using a DB 608 capillary column. In such case a detection limit of 1.0 µg/kg soil for both compounds could be achieved for a soil sample size of 50 g. This showed the importance of verifying the results of GC/ECD analysis with two capillary columns of different polarities.

The results of soil fortification experiments are shown in **Table 5.9**. In the concentration range of 5 - 50 µg/kg soil, average recoveries of triallate were 92 ± 13 % for the loamy sand soil and 86 ± 8 % for the silty sand soil. Average recoveries of TCPSA were 58 ± 10 % and 71 ± 6 % for the two soil types, respectively.

Table 5.9 Recoveries (%) with standard deviation of triallate and its metabolite TCPSA from 50 g of spiked soil samples (GC/ECD analysis with DB-5 capillary column).

Soil	Spiking level [µg/kg soil]	Recovery of triallate [%]	Recovery of TCPSA [%]	Replicates [n]
loamy sand	5.0	100 ± 12	57 ± 5	5
loamy sand	10.0	100 ± 16	54 ± 5	5
loamy sand	20.0	84 ± 7	71 ± 6	7
loamy sand	50.0	86 ± 1	52 ± 8	3
Silty sand	5.0	90 ± 11	73 ± 6	2
Silty sand	10.0	86 ± 8	70 ± 5	3
Silty sand	50.0	82 ± 2	69 ± 5	2

Efforts have been made in this work to improve the recovery of TCPSA from soil samples which was generally lower than that of triallate and might result from one or more of the sample preparation steps involved and should be closely investigated. Breakthroughs during solid phase extraction might be responsible for the losses of TCPSA. Accordingly, breakthrough tests were carried out to verify the extraction performance of the NR_4^+-cartridge.

Duplicates of the silty sand soil and four replicates of the loamy sand soil were spiked with TCPSA standard to give a concentration level of 20 µg/kg for each sample. After methanol extraction each soil extract was passed through two stacked NR_4^+-cartridges, in order to watch whether TCPSA had possibly passed through the first extraction cartridge (breakthrough) but had been retained by the second one. Through comparing the corresponding peak areas by GC/ECD analysis, signals of TCPSA methyl ester found in the second extraction cartridge

was all less than 0,3 % of that found in the first one for both soil types. Thus, there was no evidence of any breakthroughs of TCPSA from the NR_4^+-cartridges.

Another approach was to test the efficiency of the methanol extraction. To do this, TCPSA was added to different soil samples at different preparation stages, namely before methanol extraction, before solid phase extraction, and before derivatization, respectively. The soil samples were treated as usual. The results showed that the recoveries of TCPSA from the three variations were comparable to each other. Apparently, methanol extraction or solid phase extraction steps were not responsible for the comparably lower recoveries of TCPSA in soil samples. It was much likely to be caused by interference of soil matrices during the derivatization. Indeed, using matrix standards of TCPSA methyl ester, which were prepared by adding TCPSA standards to blank soil extracts before derivatization, gave better recoveries of TCPSA from soil samples. Nevertheless, only methylated TCPSA standards were used for calibrations in this work, since the behavior of TCPSA could be well monitored without using matrix standards.

5.2.2 Degradation of triallate and formation of TCPSA

The analytical method developed for the determination of triallate and especially TCPSA in water and soil samples were validated by applying this method for the sample preparation and analysis in laboratory batch experiments, in sorption behavior studies and in laboratory lysimeter experiments.

Figure 5.15 shows experimental results of triallate degradation and TCPSA accumulation in laboratory batch experiments with triallate in doubled application rate within an incubation period of 59 days. Under the experimental conditions, degradation of triallate and formation of TCPSA occurred immediately. Triallate disappeared continuously without lag-phase from 2690 to 1550 µg/kg soil, namely 58 % of the triallate amount initially applied. The DT_{50} value was calculated to be 81 days. This is in good agreement with the investigations of Gottesbüren (1991), who found triallate half-lives varying between 57 - 85 days. 14 µg/kg soil of TCPSA was found two weeks after the triallate application. As the concentration of triallate decreased, the concentration of TCPSA increased continuously during the whole incubation period and reached an amount of 98 µg/kg soil at the end of the experiments, corresponding to about 4 % of the amount of triallate initially applied.

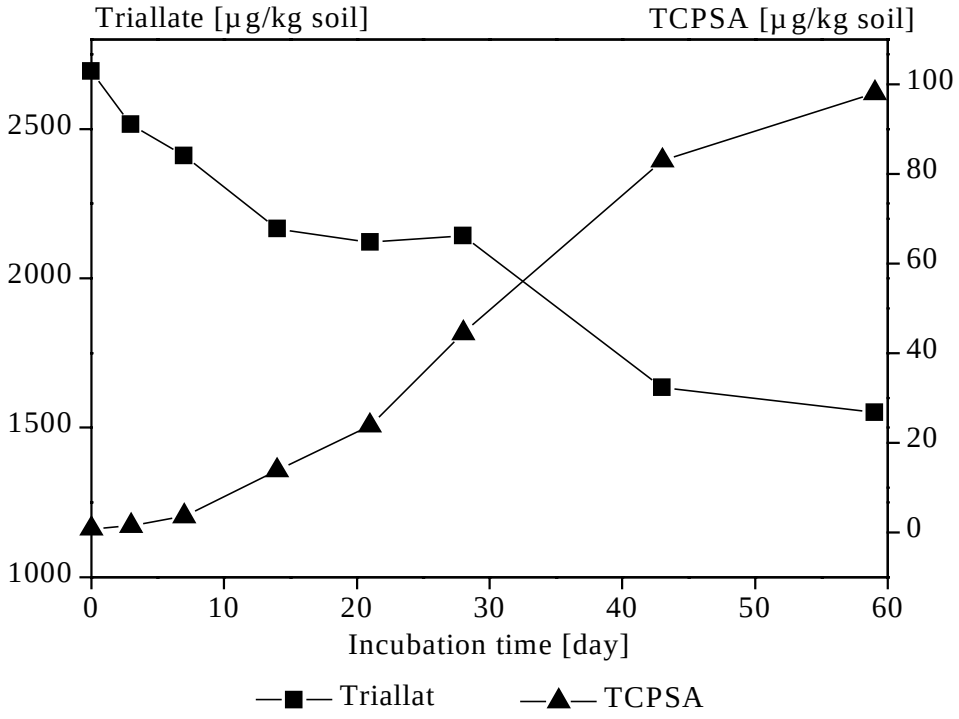


Figure 5.15 Concentrations of triallate and TCPSA formed in a loamy sand soil in the laboratory batch experiments. Samples were analyzed in duplicate.

This result was verified in another laboratory batch experiment with tripled application rate of triallate. With the higher initial concentration of triallate, TCPSA already appeared at a concentration level of 5.5 µg/kg soil one week after the incubation. Its concentration increased successively to 159 µg/kg soil within the incubation period of 53 days, accounting also for 4 % of the amount of triallate initially applied, whereas triallate residues continuously reduced during the experiment period.

The results of these laboratory batch experiments confirmed that degradation processes contributed in the counteracting of triallate accumulation in soil. TCPSA is an important degradation product of triallate degradation in soil. It seemed to be quite stable in soil, or its further transformation occurred much slower than its formation, resulting in its accumulation in soil, at least within the investigation periods in this work. **Figure 5.16** shows a typical chromatogram of a soil sample from laboratory batch experiments, in which 31 µg TCPSA/kg soil was determined by GC/ECD analysis.

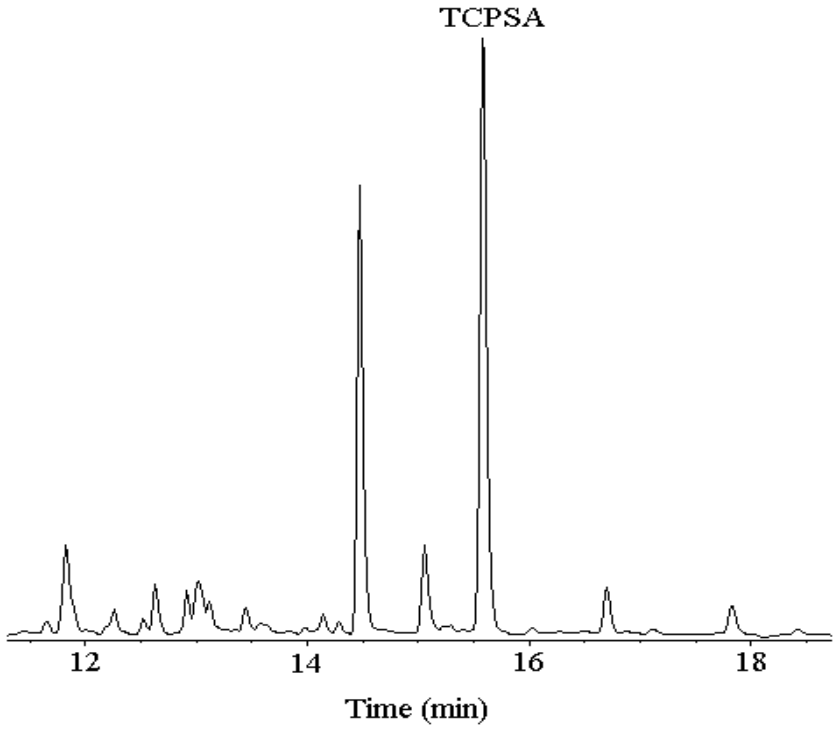


Figure 5.16 Chromatogram of a soil extract from a laboratory batch experiment. TCPSA formed as triallate degradation was determined to be 31 µg/kg soil.

The formation of TCPSA as triallate degradation product was confirmed by GC/MS analysis. TCPSA was identified in soil samples from laboratory batch experiment through comparing the GC retention time and the EI and NCI mass spectra of these samples with those of methylated TCPSA standards. In addition, duplicates of blank soil samples without the spiking of triallate was investigated as well. Although a small blank signal was detected at the retention time of TCPSA methyl ester, it showed a completely different mass spectrum. The blank signal was, therefore, caused by interference of an unknown coextractant from the soil matrix. These results from GC/MS analysis proved that the formation of TCPSA was from triallate degradation in soil.

5.2.3 Soil sorption of triallate and TCPSA

The results listed in **Table 5.10** reveal the significant difference in soil sorption behavior between triallate and its metabolite TCPSA. With a K_d value of 102 only about 1 % triallate was determined in the soil solution. This means nearly 99 % of triallate originally added

Table 5.10 Distribution coefficients (K_d values) of triallate and its metabolite TCPSA between soil and soil solution.

Substance	Spiking level [µg/50 g soil]	K_d value	Replicates [n]
Triallate	66	102 ± 11	4
TCPSA	5.0	0.02 ± 0.02	4

remained adsorbed on the loamy sand. This result is in good agreement with the investigations of Smith (1969, 1970), who studied the sorption and leaching of triallate and reported that triallate was adsorbed, according to the soil properties, to soil from aqueous solutions to an extent of 79-96 %. Therefore, triallate tended to be adsorbed in topsoil and would not be transferred into deep soil layers by leaching. In addition, the high K_d value of triallate indicates not only reduced leaching potential but also low dissipation rate, because dissipation rate depends strongly on the quantity of the pesticide dissolved in the soil solutions (Anderson, 1984, 1981). Triallate is generally regarded as persistent. This could be interpreted as the result of strong adsorption of this herbicide to soil matrix. Adsorption reduced its concentration in the soil solution, resulting in diminished disappearance from soil.

In remarkable contrast, almost all the TCPSA originally added was found remaining in the soil solution. The K_d value was only 0.02 for TCPSA in the loamy sand from the investigation site Sickte. With its poor soil sorption ability and its high water solubility, TCPSA possess a significant greater leaching potential than its parent compound. Thus, triallate sorption in topsoil and TCPSA transport into deeper soil layers would be expected as the main concentration determining processes.

Negative influences of pesticides or their degradation products on ground water could be expected, when the following criteria were fulfilled: water solubility > 30 mg/L; soil/water distribution coefficient (K_d value) < 10; DT_{50} value in soil > 21 days (BBA, 1990). Thus, negligible leaching potential of triallate could be derived for the loamy sand soil from the investigation site Sickte, whereas potential ground water contamination might be caused as a result of TCPSA transport and accumulation under the root zone. The great difference in the leaching potential of the parent compound triallate and its polar metabolite TCPSA also

highlighted the importance of simultaneous investigation on the behavior of pesticides and their corresponding degradation products.

It is well known that distribution coefficients (K_d values) are normally not sufficient for the prediction of sorption behavior and leaching potential of pesticides or their metabolites in soils (Kookana and Aylmore, 1993; Scheunert, 1993), because K_d values are measured under the presumption that static adsorption-desorption equilibrium could be rapidly reached in a simplified system. This presumption is, however, far away from the natural fields conditions, where adsorption-desorption reactions are dynamic processes.

5.2.4 Leaching of triallate and TCPSA

With regard to the shortages of soil/water distribution coefficients (K_d values), laboratory lysimeter experiments with undisturbed soil monoliths is a better alternative for the investigation of degradation, sorption and transport of pesticides and their metabolites in soil. These lysimeter experiments are designed to be carried out under experimental conditions simulating the field dynamic conditions. Therefore, for a better evaluation of the adsorption and leaching behavior of triallate and TCPSA, laboratory lysimeter experiments were carried out in this work.

5.2.4.1 Breakthrough of triallate and TCPSA in percolates

Figures 5.17 - 5.19 depict the breakthrough curves of triallate and TCPSA in the laboratory lysimeter experiments I - III. Before the applications of triallate and TCPSA, no triallate or TCPSA was found in the soil samples. The determination of TCPSA in the percolates from lysimeter I and lysimeter II proved the degradation of triallate as well as the formation and leaching of TCPSA in the lysimeter experiments with or without pre-incubation. Under the experimental conditions, degradation of triallate and formation of TCPSA took place immediately. One week after the experiment was started, 0.12 µg TCPSA/L was found in the percolate from lysimeter I.

As shown in **Figures 5.17 and 5.18,** triallate was detected in all percolate samples from lysimeter I and lysimeter II, indicating its breakthroughs throughout the lysimeter experiments.

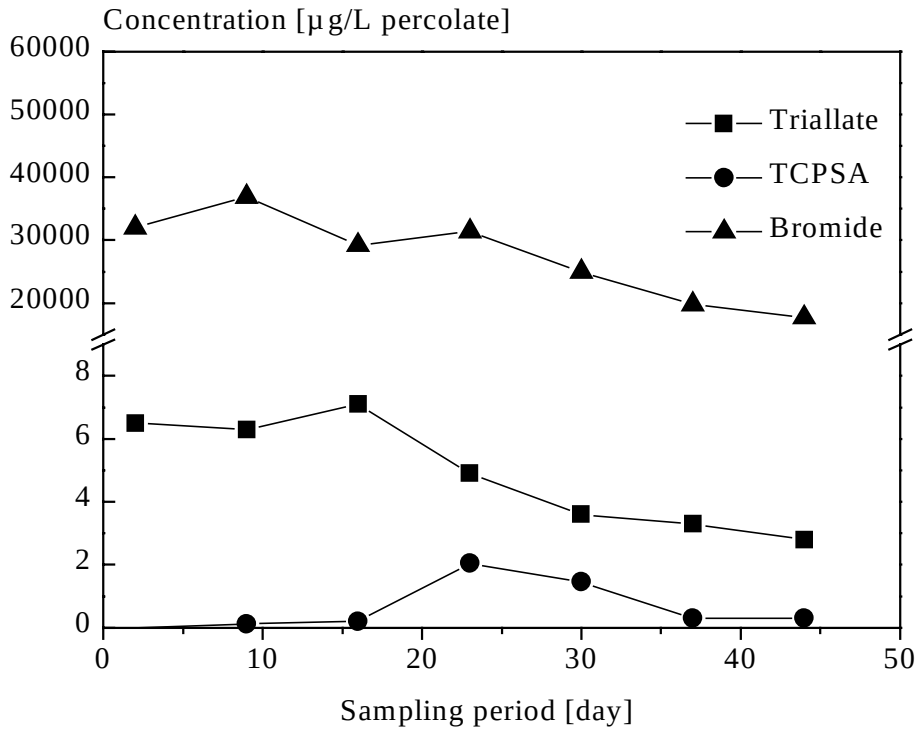


Figure 5.17 Breakthrough curves of triallate, TCPSA and bromide in lysimeter experiment I.

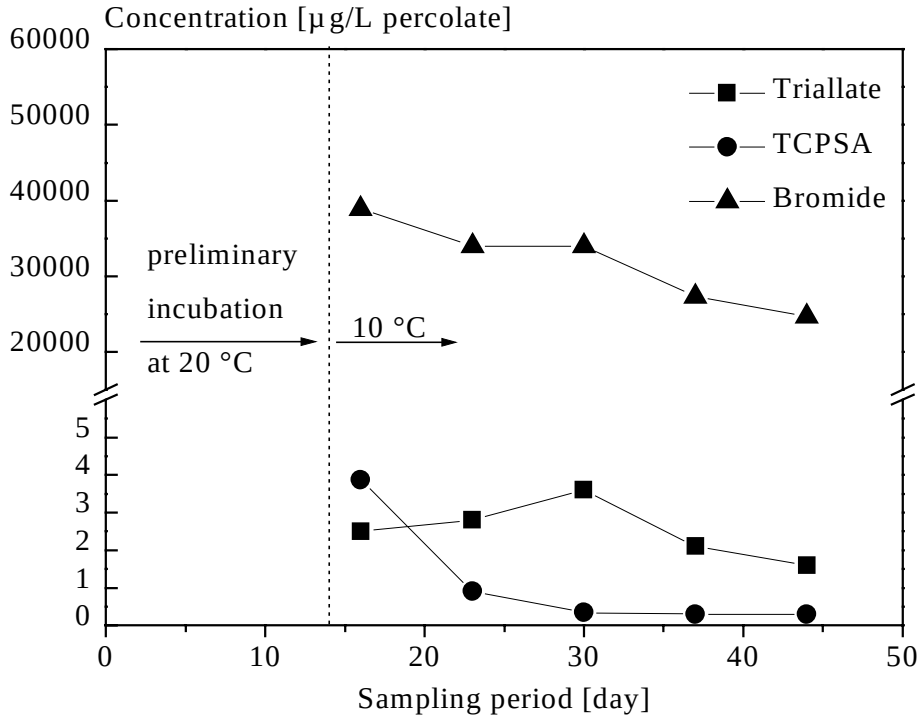


Figure 5.18 Breakthrough curves of triallate, TCPSA and bromide in lysimeter experiment II.

During the first three times of sprinkler irrigation, the breakthrough of triallate at concentration levels of 6.3 - 7.1 µg/L percolate from lysimeter I (**Figure 5.17**) appeared, especially, quite high. This rapid breakthroughs of triallate with infiltration water might be explained as the consequence of the so called preferential flow effect, resulting from macropores in the soil structure. This was confirmed by the bromide tracer detected in percolates. This kind of phenomenon has been reported in a number of studies (see **Chapter 1.2**) but is difficult to be described quantitatively because of its complicate and variable nature. Due to preferential flows, leaching potential of triallate could strongly increased. The reduced breakthroughs of triallate in the later stage of the experiments might be explained as a result of declined preferential flow effect, as interactions between triallate and the soil matrix became stronger with the aging. This was also confirmed by the experimental results of lysimeter II. After the pre-incubation, adsorption of triallate in soil increased and breakthroughs of triallate through preferential flow effect reduced evidently (**Figure 5.18**). However, total amounts of triallate released from the soil monoliths of lysimeter I and lysimeter II were only 0.14 % and 0.06 % of the initial amount of triallate applied, respectively.

As depicted in **Figure 5.17,** no TCPSA was found in the percolate from lysimeter I after the first irrigation cycle. Then, breakthroughs of TCPSA formed from triallate degradation increased to a maximum of 2.0 µg/L, before it reduced to a nearly constant level of 0.30 µg/L in the last two weeks of the lysimeter experiments. The breakthroughs of TCPSA from lysimeter II was different from that of lysimeter I at the early experimental stage, as shown in **Figure 5.18**. Directly after the preliminary incubation, the highest breakthrough concentration of TCPSA at 3,9 µg/kg was determined in the first percolate sample. Then, the concentration rapidly decreased to a nearly constant level of 0.30 µg/L in the last three weeks that is similar to the result of lysimeter I at the later experimental stage. These concentration declines of TCPSA in the percolates from lysimeter I and lysimeter II should be caused by its stronger retentions on the soil matrix with the aging.

A similar situation happened in lysimeter III (**Figure 5.19)**. TCPSA concentration in percolates from lysimeter III increased first to a maximum of 116 µg/L. Then, TCPSA concentration in the percolates decreased with the time and approached a constant value of 30 µg/L percolate which was, however, still 100 times higher than that of triallate. Thus, in

comparison with the leaching behavior of the parent compound in lysimeter I and lysimeter II, the much

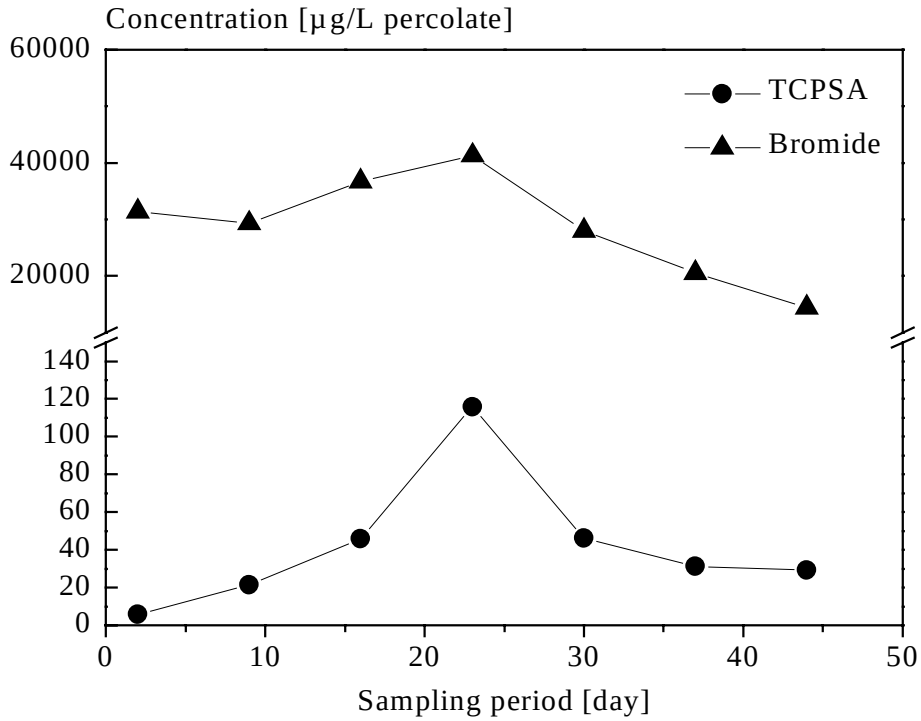


Figure 5.19 Breakthrough curves of TCPSA and bromide in lysimeter experiment III.

higher concentrations of TCPSA in the percolates from lysimeter III indicated a much higher leaching potential of this metabolite, when both compounds were applied to the same soil under same experimental conditions. This result reflects the assumption derived from the K_d values of triallate and TCPSA..

5.2.4.2 Distribution of triallate and TCPSA in soil monoliths

After the lysimeter experiments were finished , distribution of triallate and TCPSA in soil was evaluated through the analysis of the soil monoliths differentiated in depths.

The distribution pattern of triallate in the soil monoliths, as shown in **Figure 5.20**, indicated that triallate applied to lysimeter I and lysimeter II was strongly adsorbed to soil matrix. The highest triallate concentrations of 2080 and 1759 µg/kg soil, equivalent to 94 % and 92 % of

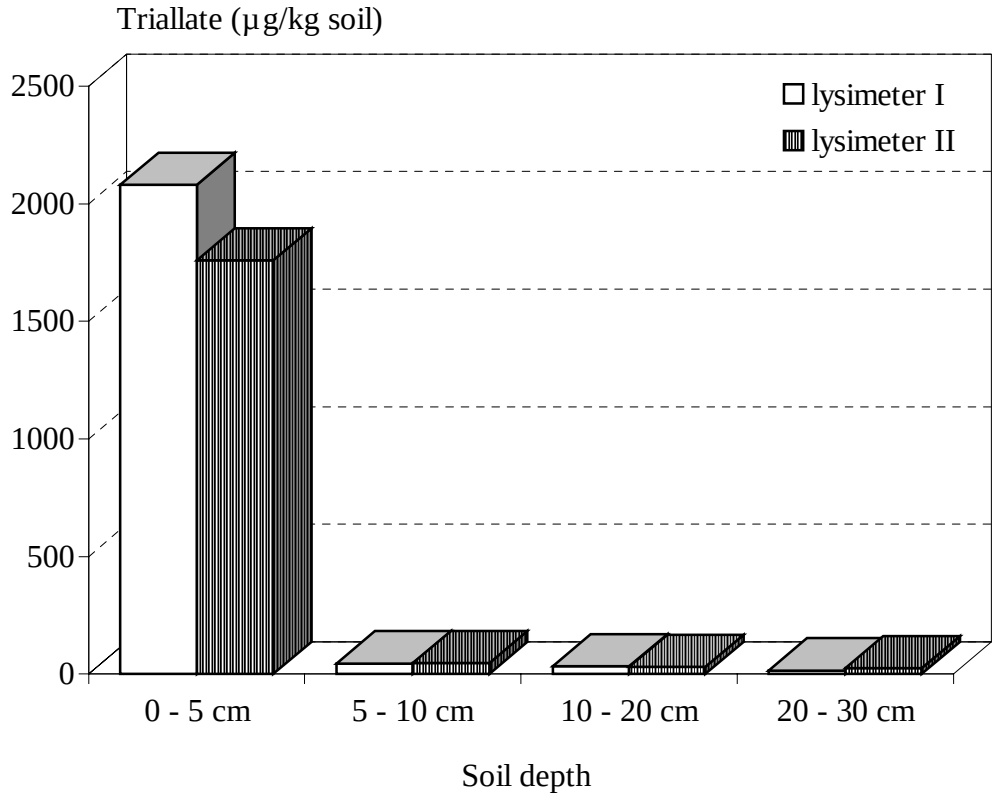


Figure 5.20 Distribution of triallate in soil layers after sprinkler irrigation

the extractable triallate residues in lysimeter I and lysimeter II, respectively, were found in the superficial 0-5 cm layer. In the 5-10 cm soil layer the concentrations sharply dropped to 46 and 47 µg/kg soil, respectively. These results confirm that triallate is relatively immobile and mainly remains in the superficial 0-5 cm soil layer as reported by Gottesbüren et al. (1992) and Smith (1969, 1970). With increasing soil depths triallate concentrations substantially decreased to 16 and 25 µg/kg soil in the 20-30 cm soil layer.

As shown in **Table 5.11**, the total amounts of triallate found in percolates, recovered in soil monoliths, as well as degraded to TCPSA accounted together for only 66 and 54 % of the amount of triallate initially applied to the lysimeter I and lysimeter II, respectively. Further losses of triallate might be caused by volatilization, degradation to other metabolites, mineralization and formation of non-extractable residues.

Table 5.11 Triallate, TCPSA and bromide in percolates and soil by laboratory lysimeter experiments.

Applied amount	Lysimeter I 5200 µg triallate		Lysimeter II 5200 µg triallate		Lysimeter III 5000 µg TCPSA	
	Percolate [µg]	Soil [µg]	Percolate [µg]	Soil [µg]	Percolate [µg]	Soil [µg]
Triallate	7.3	3281	2.9	2687	--	--
TCPSA	0.5	130	0.8	145	76.1	2567
Bromide*	34800	-/-	30500	-/-	53400	-/-

* applied amount to each lysimeter: 65 mg

-- not applied; -/- not analyzed

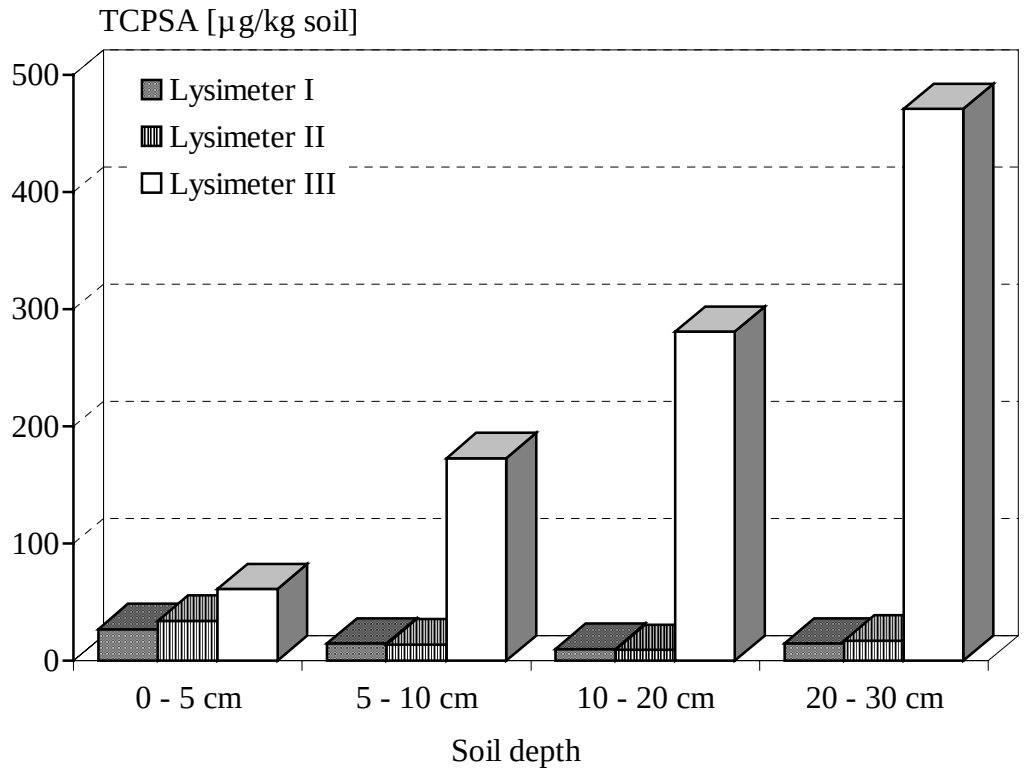


Figure 5.21 Distribution of TCPSA in soil layers after sprinkler irrigation

Distribution patterns of TCPSA in the soil monoliths of lysimeters I - III are shown in **Figure 5.21**. The distribution of applied TCPSA in the soil monolith of lysimeter III presents a striking contrast to the distribution of the parent compound triallate. The concentrations of TCPSA increased with the soil depths. Over 57 % of TCPSA recovered from the soil samples appeared in the lowest 20-30 cm soil layer. This result confirmed the high leaching potential of this metabolite.

TCPSA was also found in all soil layers of lysimeter I and lysimeter II. The distributions of TCPSA formed as triallate degradation product in the soil monoliths of these two lysimeters were similar to each other, but different to the distribution of TCPSA directly applied to the soil monolith of lysimeter III. The highest concentrations of TCPSA were within the superficial 0-5 cm soil layer in lysimeter I and lysimeter II, whereas concentrations in the 5-10 cm, 10-20 cm and 20-30 cm soil layers were relatively constant. The high concentrations of TCPSA formed in the superficial soil layers corresponded to the high concentrations of the parent compound in the superficial 0-5 cm soil layers.

To Compare the amounts of TCPSA found in percolates with those recovered in soil samples (**Table 5.11)**, it is clear that only very small fractions, namely 0.4 - 1.5 %, of TCPSA formed or applied were released with infiltration water from lysimeters I - III. These unexpected low concentrations of TCPSA in the percolate samples seemed to be contrary to the extreme low K_d value of 0.02 determined for TCPSA in the batch equilibrium study, in which TCPSA remained almost completely in the soil solution. This apparent disagreement is likely due to the differences in the experimental conditions. It showed that transport of a pesticide or its degradation products in soil is a very complex process. Besides the specific substance and soil properties, soil structures and other environmental factors affect the pesticide leaching behavior, as well (see **Chapter 1.2**). The method for the determination of soil/water distribution coefficient (K_d value) does not take into consideration of many of these factors. In addition, some experimental conditions for the determination of K_d values, like shaking and centrifuging, do not exist in the real field conditions. This has probably led to the overestimation of TCPSA leaching. A similar result was reported for fenpropimorphic acid which was formed in soil via oxidation of the tertiarybutyl group of the morpholine fungicide fenpropimorph. High leaching potential of fenpropimorphic acid could be derived from its low K_d values of 1.0 and 0.5 in clayey silt and silty sand soils. But no significant transport of

this acid into deeper soil layers was found in lysimeter experiments and field studies (Ebing et al., 1995; Stockmaier, et al., 1996).

However, these small amounts of TCPSA leaching into deeper soil layers might be still significant for a potential ground water contamination. In the laboratory batch experiments TCPSA was proven to be relatively stable in the soil environment, showing considerable accumulation tendency. Additionally, with regard to the significant lower microbial degradation ability of soils in deeper layers, a concentration pool of the very polar metabolite TCPSA might be built as the result of a long-term leaching effect of TCPSA. It is worth to mention that although some persistent pesticides such as s-triazines and their metabolites were recognized as immobile in many research works, they have also been often found in deeper soil layers and in ground water zone under field conditions, as reviewed by Dörfler et al. (1997). Wehtje et al. (1984) have pointed out that leaching of very small amount of s-triazines, e.g. less than 0.1 % of the amount applied, may still lead to ground water contamination. Therefore, a potential ground water contamination by TCPSA could not be excluded.

As discussed above, triallate concentrations in the superficial soil layers of lysimeter I and lysimeter II were 2080 and 1759 µg/kg soil, respectively. This concentration difference reflected the temperature influence of preliminary incubation on triallate degradation. Except the lower triallate residues, higher TCPSA concentrations were determined in the superficial soil layer of lysimeter II, as shown in **Table 5.11**. These results appeared to be also supported by the results of a simultaneous supplementary batch experiment, as depicted in **Figure 5.22**.

Within the preliminary incubation period at 20 °C, triallate concentration continuously decreased, while TCPSA concentration steadily increased. Then, the rates of TCPSA formation and triallate degradation leveled off as a result of the shift of incubation temperature from 20 °C to 10 °C. This effect was apparently due to the reduced degradation activity of microorganisms in soil as the temperature reduced.

In addition, the total amount of TCPSA determined in soil samples from lysimeter I and lysimeter II were 130 and 145 µg, equivalent to concentrations of 66 and 74 µg/kg soil, respectively, which is in good agreement with the end concentration of TCPSA formed in the

supplementary batch experiments, in which concentrations of triallate residue and TCPSA formed were determined as 2292 and 66 µg/kg soil after 45 days of incubation.

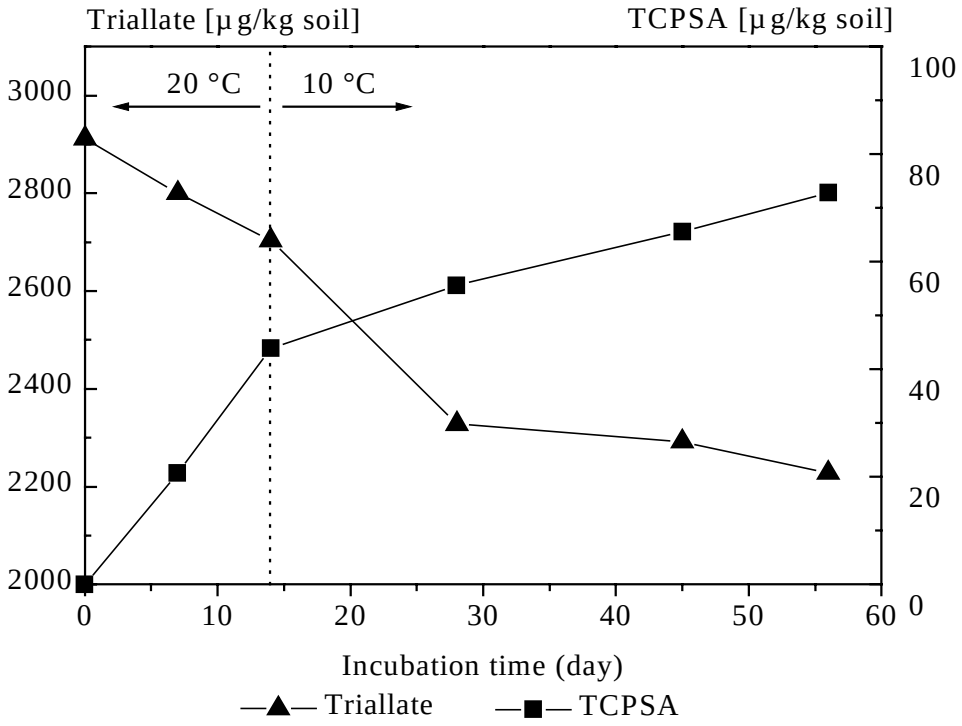


Figure 5.22 Triallate degradation and TCPSA accumulation in the supplementary batch experiment. Samples were analyzed in duplicates.

In all the lysimeter experiments, bromide was applied as a conservative tracer for monitoring the water flow. Approximately constant breakthrough curves of bromide showed nearly homogeneous structures of the soil monoliths. Recoveries of this tracer varied from 47 - 82 %, indicating that the total volume of irrigation water used in the lysimeter experiments was too low for a total release of the tracer bromide.

5.2.4.3 Adsorption of TCPSA in soil monoliths

As mentioned above, in contradiction with its soil/water distribution coefficients (K_d value), TCPSA released with infiltration water from lysimeters I - III accounted for only 0.4 - 1.5 % of its amounts formed as triallate degradation product or directly applied to the soil monoliths. The predominant parts remained in the soil. However, with its high polarity and water solubility, TCPSA was most likely to be solved in the soil solution than to be adsorbed to the

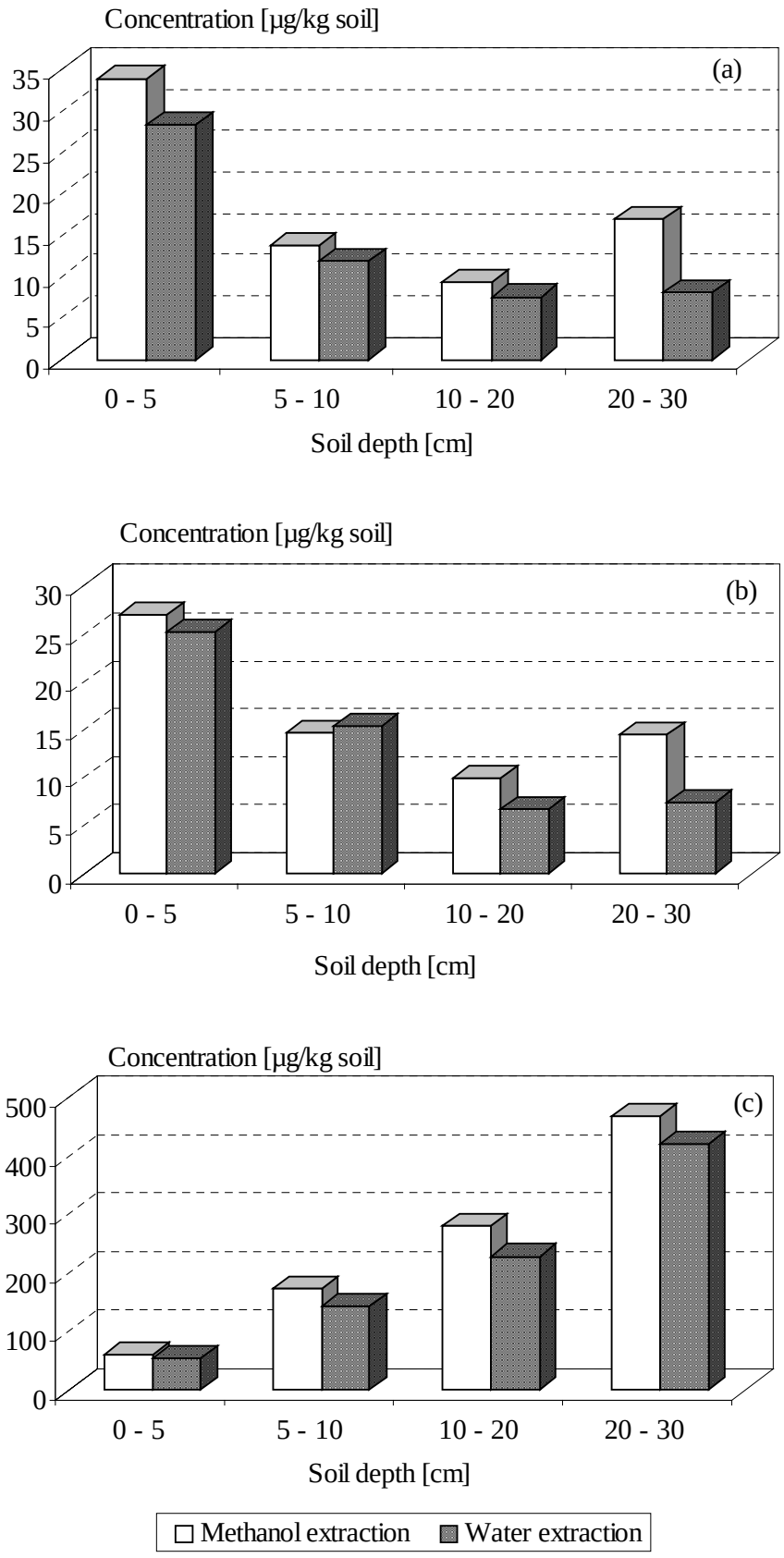


Figure 5.23 Comparison of the extraction of TCPSA in soil with methanol and water, (a) - (c): soil monoliths I - III.

soil matrix. In order to estimate whether further irrigation would continuously release these adsorbed amounts, it was of great interest to find out to which extent and strength TCPSA was really adsorbed to the soil matrix. Therefore, soil samples were also extracted with water instead of with methanol. The extraction was carried out in simulation of the experimental conditions used for the determination of K_d values.

The results are shown in **Figures 5.23a - 5.23c** and compared with the results of methanol extractions. Recoveries of TCPSA from water extractions were between 50 % and 104 % of the recoveries from methanol extractions, with an average value of 80 ± 16 %. These results showed that TCPSA was only weakly adsorbed to the soil matrix, no matter it was directly applied to the soil or formed by triallate degradation in the soil. Under the mechanical effects like shaking and centrifuging employed by the methanol or water extractions in this work, the weak adsorption of TCPSA on the soil matrix could be easily disrupted. Hence, the seemingly contradicting high concentrations of TCPSA in the soil monoliths might be elucidated as a consequence of soil solutions trapped in the soil matrix. Soil solutions have been described to be held to the soil matrix in three major ways: as capillary films around individual soil particle, as capillary wedges in small spaces between soil particles, and as imbibed water incorporated into the colloidal structure of particles of clay and organic matters (Turk et al., 1984). In the third possibility, soil solutions become relatively tightly bound up in the soil matrix. Infiltration water draining through soil monoliths is likely not able, or at least not easy, to carry away TCPSA solved in the soil solutions that are tightly hold by soil matrix. However, this kind of adsorption should be reversible and undergoes constant changes. Therefore, under natural dynamic conditions in the field, TCPSA might become available again for further transport into deeper soil layers by infiltration water in a long-term, resulting in potential ground water contamination.

In contrast, no triallate was found in any water extracts. Consequently, the breakthrough of triallate should be mainly due to preferential flows and is only a short-term phenomenon. As the effect of preferential flows reduced with the time, less and less triallate would be expected to be transported into deeper soil layers by infiltration water.

6 Summary

For a better understanding of the environmental impacts of pesticides it is of importance to investigate not only the fate of the active substances, but also the formation and behavior of their degradation products. The degradation behavior of the carbamate insecticide pirimicarb and the thiocarbamate herbicide triallate in soils was investigated in this work, with emphasis on the formation of their corresponding metabolites.

Pirimicarb-desmethylformamido (PMC-DF) and pirimicarb-desmethyl (PMC-D) have been chosen as representative metabolites of pirimicarb and were studied in this work. For a simultaneous sensitive determination of pirimicarb, PMC-DF and PMC-D in soil, the DFG S19 multi-residue method was adapted. Using this method, determination limits of 10 µg/kg soil by HPLC/UV or GC/MS analysis and 5 µg/kg soil by selective GC/NPD analysis were achieved for all three compounds.

Laboratory batch experiments were performed to study the fate of pirimicarb in a clayey silt soil and a silty sand soil from the investigation sites Neuenkirchen (NK) and Nienwohlde (NW), respectively. The degradation pattern of pirimicarb showed remarkable differences in the two soil types. Pirimicarb degraded at a moderate rate in the clayey silt soil and a DT_{50}-value of 16 days as well as a DT_{90}-value of 52 days could be derived, whereas it exhibited surprisingly high persistence in the silty sand soil with a DT_{50}-value of 213 days. The differences in pirimicarb persistence could be ascribed to the different inherent properties of the two soil types, in which pirimicarb had stronger acid-base interactions with the acidic silty sand soil. As a consequence, pirimicarb showed stronger sorption to the soil matrix and decreased concentration in the soil solution. That resulted in diminished bioavailability and high persistence of pirimicarb in the silty sand soil. This has been proven by adsorption equilibrium studies which were performed to evaluate soil/water distribution coefficients (K_d values) of pirimicarb and its metabolites. The K_d value of pirimicarb was 46 in the silty sand soil and 2.0 in the clayey silt soil.

Laboratory batch experiments confirmed the formation of the metabolites PMC-DF and PMC-D. In the clayey silt soil, the two metabolites increased to maximum concentrations of 133 and 71 µg/kg soil, respectively, and then decreased to 83 and 32 µg/kg soil as a result of

further degradation. In the silty sand soil, concentrations of the two metabolites increased to about 30 and 25 µg/kg soil and remained nearly constant until the end of the experiments. While the K_d-values of PMC-DF were comparable in the two soil types, the K_d-value of PMC-D, a secondary amine, was over one magnitude greater in the acidic silty sand soil than in the clayey silt soil, similar to the sorption behavior of the parent compound pirimicarb.

Field experiments with doubled pirimicarb application rate were carried out at the two investigation sites NK and NW. In contrast to the laboratory batch experiments, pirimicarb disappeared very fast in the first week after application at both investigation sites. Then, the dissipation rates sharply slowed down and the concentration of pirimicarb remained nearly constant until the end of the experiments. The concentration changes of pirimicarb could be characterized with DT_{50}-values of 1 and 3 days and DT_{90}-value of 18 and 26 days, respectively, in NK and NW. Photo-degradation might have played an important role in the rapid disappearance of pirimicarb at the early stage of the experiments. Accelerated microbial degradations due to the high summer soil temperatures could also have contributed to the observed concentration decreases of pirimicarb. The reduced dissipation rates of pirimicarb in the later experimental stage should be resulted from the stronger adsorption of pirimicarb residues to the soil matrices with the time. Although the determination of pirimicarb in 5-10 cm soil layers confirmed its vertical movement, no differences could be observed between the two soil types as would be expected with the great difference in pirimicarb K_d-values in these two soil types. The formation of PMC-DF and PMC-D were also verified in the field experiments. Their concentrations were generally lower than those in laboratory batch experiments, indicating the contribution of photo-induced degradation to the further transformation of the two metabolites

2,3,3-trichloro-prop-2-en-sulfonic acid (TCPSA) was chosen in this work for characterizing the degradation of triallate in soil. In remarkable contrast to the non-polar parent compound, TCPSA is very polar and water soluble. For a sensitive determination of triallate and TCPSA, a new method based on solid phase extraction was developed in this work.

Soil samples were extracted with methanol and diluted with water to yield a methanol/water ration of 1:4. Triallate was retained on C_{18}-cartridges, while TCPSA was enriched on quaternary amine (NR_4^+) anion exchange cartridges. triallate was eluted with methanol/ethyl acetate (1:1) and TCPSA with 0.5% sulfuric acid/methanol mixture (v:v). Subsequently,

TCPSA was methylated with trimethyl orthoformate to facilitate the GC/ECD analysis. Using this method, a determination limit of 5 µg/kg soil was reached for both triallate and TCPSA. The recoveries of triallate were 92 ± 13 % for a loamy sand soil and 86 ± 8 % for a silty sand soil. Recoveries of TCPSA were 58 ± 10 % and 71 ± 6 % for the two soil types, respectively. For the analysis of water samples, the determination limit was 0.05 µg/L for both compounds, with recoveries of 84 ± 14% for triallate and 100 ± 22 % for TCPSA.

Compared with conventional solvent extraction methods reported in literature, the method developed in this work is significantly more effective with considerably less solvent consumption and remarkably shorter sample preparation time.

Laboratory batch experiments clearly proved the formation of TCPSA as a degradation product of triallate in the loamy sand soil. Triallate disappeared steadily without lag-phase, with a DT_{50}-value of 81 days. TCPSA appeared to be stable in the soil. At least its further transformation rate was slower than its formation rate. Concentration of TCPSA increased during the whole soil incubation period and accounted for about 4 % of the amount of triallate initially applied.

Sorption studies were carried out to estimate the leaching tendency of triallate and TCPSA. The K_d-values determined in the adsorption equilibrium studies were 102 for triallate and 0.02 for TCPSA in the loamy sand soil, revealing significant higher mobility of the polar metabolite. This was confirmed by laboratory lysimeter experiments, in which triallate and TCPSA were applied to undisturbed soil monoliths. Triallate was strongly adsorbed in the superficial 0-5 cm soil layers, whereas TCPSA was mainly transported to the 20-30 cm soil layers. Preferential flow phenomenon can be inferred for triallate at the early stage of the lysimeter experiments. Most of TCPSA formed as triallate degradation product or directly applied were remained in the soil. Only 0.4 - 1.5 % were determined in the percolates. This seemed to be contrary to the leaching potential revealed by its low K_d-value, but could be explained as a result of different experimental conditions between the sorption equilibrium studies and the laboratory lysimeter experiments. This assumption was confirmed by water extractions of soil samples which clearly showed the weak sorption of TCPSA to the soil matrix. Thus, in a long-term, TCPSA could be released from the soil matrix and further transported into deeper soil layers with infiltration water under field conditions, resulting in a potential ground water contamination.

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